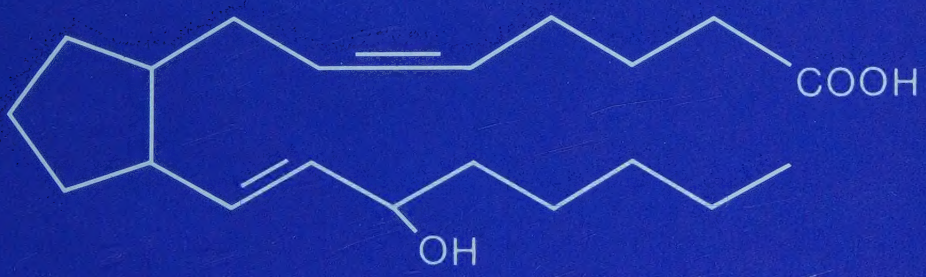
Tore Uski

PROSTANOIDS AND CEREBRAL ARTERIES

Studies on receptor characterization and excitation – contraction coupling



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Abstract
The effects of prostanoids were studied on isolated feline and human cerebral arteries. Based on the orders of relative potencies, prostaglandin (PG) -induced cerebrovascular contraction in both species can be interpreted as being mediated by a TXA_2-sensitive receptor, whereas relaxant responses probably are mediated via a receptor sensitive to PGI_2. The effects of PG's may differ between species. PGI_2 relaxed human arteries even if these were contracted by potassium (K^+), but no such effects were observed in feline vessels. These were, however, relaxed by PGI_2 if contracted by e.g. $PGF_{2\alpha}$.

From experiments with calcium-free medium, it was concluded that $PGF_{2\alpha}$-induced contractions in cerebral arteries are relatively independent of free extracellular calcium. Instead, these apparently are mediated mainly by the release of cellularly bound calcium. By comparing the effects of calcium-readdition to vessels exposed to various combinations of $PGF_{2\alpha}$, K^+ and nifedipine, it could be concluded that $PGF_{2\alpha}$ promotes transmembrane calcium-influx via pathways which seem at least partially different from potential sensitive calcium channels. These pathways probably represent receptor operated channels.

Despite the fact that $PGF_{2\alpha}$-induced contractions are relatively independent of free extracellular calcium, these can be efficiently counteracted by the calcium channel blocker nifedipine. Different methods of affecting cellular calcium (EGTA, sialidase, caffeine), as well as means of blocking (nifedipine, diltiazem, manganese) and enhancing (BAY K 8644) calcium entry were employed in order to obtain information on the localization of the calcium-stores mediating $PGF_{2\alpha}$-induced contractions. The results suggest that these contractions are mainly mediated by the release of calcium from the outside of the cell membrane, which may explain the divergent findings with calcium entry blockers and calcium-free medium.

Key words
Feline and human pial arteries, prostanoids, prostanoid receptors, species differences, calcium-free medium, calcium entry blockers, caffeine, sialidase, EGTA, membrane-bound calcium

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Studies on receptor characterization and excitation - contraction coupling

By
Tore K Uski

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Cover: Structure formula of the bis-enoic prostaglandin skeleton

PREFACE

This thesis is based on the following articles:

I. Uski, T., Andersson, K.-E., Brandt, L., Edvinsson, L., Ljunggren, B. 1983. Responses of isolated feline and human cerebral arteries to prostacyclin and some of its metabolites. J. Cereb. Blood Flow Metabol. 3:238-245.

II. Uski, T.K., Andersson, K.-E. 1984. Effects of prostanoids on isolated feline cerebral arteries. I. Characterization of the contraction-mediating receptor. Acta Physiol. Scand. 120:131-136.

III. Uski, T.K., Andersson, K.-E. 1984. Effects of prostanoids on isolated feline cerebral arteries. II. Roles of extra- and intracellular calcium for the prostaglandin $F_{2\alpha}$-induced contraction. Acta Physiol. Scand. 120:197-205.

IV. Uski, T.K., Andersson, K.-E., Brandt, L., Ljunggren, B. 1984. Characterization of the prostanoid receptors and of the contractile effects of prostaglandin $F_{2\alpha}$ in human pial arteries. Acta Physiol. Scand. (In press).

V. Uski, T.K. 1984. Cellular calcium and the contraction induced by prostaglandin $F_{2\alpha}$ in feline cerebral arteries. Br. J. Pharmac. (Submitted).

VI. Uski, T.K., Andersson, K.-E. 1984. Some effects of the calcium promotor BAY K 8644 on feline cerebral arteries. Acta Physiol. Scand. (Submitted).

The articles will be referred to in the text by their Roman numerals.

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Prostaglandins

Since the discovery of prostaglandins (PG´s) about half a century ago (von Euler 1934-1937), a large number of PG´s and related substances have been described (Coceani & Pace-Asciak 1976). In the last decade, the arachidonic acid (AA) metabolic pathways via the cyclic prostaglandin endoperoxides PGG_2 and PGH_2 to thromboxanes (TX´s) (Hamberg et al. 1974, Hamberg & Samuelsson 1974, Hamberg et al. 1975), as well as the prostacyclin (PGI_2) pathway (Moncada et al. 1976 a and b) have been elucidated. Recently, yet another group of substances, the leukotrienes (LT´s), derived from the PG-precursor AA have been described (Murphy et al. 1979, Samuelsson et al. 1979-80). Fig 1 summarizes the metabolic pathways from AA which so far have been mapped out with an emphasis on the PG-cascade.

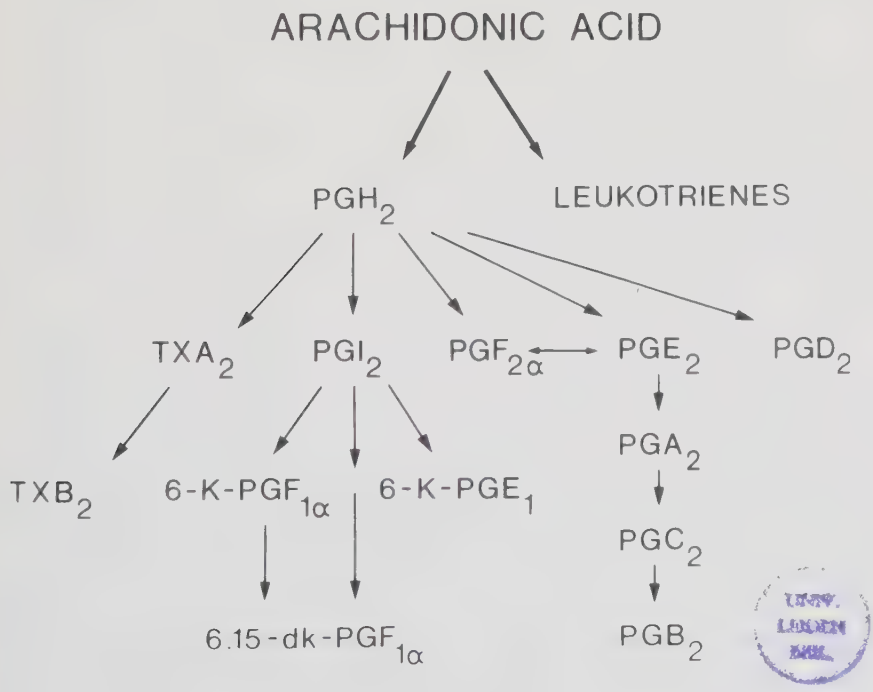

Fig 1: Schematic drawing of the metabolic pathways of the arachidonic acid cascade.

The enzyme cyclooxygenase initiates the production of prostanoids via the the PG- and TX-cascades (Coceani & Pace-Asciak 1976), whereas the synthesis of LT´s and other substances such as 12-HETE are initiated by lipoxygenase (Samuesson et al. 1980). AA serves as substrate for cyclooxygenase and is precursor for the bis-enoic PG´s (2-series), whereas dihomo-linolenic acid is the precursor for PG´s of the 1-series, and eicosa-pentaenoic acid for the 3-series of PG´s (Moncada & Vane 1978).

Prostaglandins and cerebral vasoregulation

Prostaglandins are synthezised by cerebral vessels (Hagen et al. 1979, Abdel-Halim 1980, Gerritsen et al. 1980, Goehlert et al. 1981, Gecse et al. 1982) as well as by brain tissue (Holmes & Horton 1968, Coceani & Pace-Asciak 1976, Abdel-Halim 1980, Galli et al. 1980, Ellis et al. 1982). Measurable amounts of several of these substances occur in the cerebrospinal fluid (CSF) (Holmes 1970, Abdel-Halim 1980, White et al. 1983), although measurements of the CSF-concentrations probably underestimates the true concentrations at the effector sites in the vessels (Pickard et al. 1975, Bito et al. 1976, Hagen et al. 1977). PG´s may be involved in the normal regulation of cerebral blood flow, as well as in the pathogenesis of cerebral vasospasm (White 1979, Pickard 1981, White & Hagen 1982) and migraine (Parantainen & Vapaatalo 1983, White et al. 1983).

Numerous investigations have been performed in order to study the effects of PG´s and related substances on the cerebral vasculature *in vivo* and *in vitro* (for review, see Pickard 1981, White & Hagen 1982). Studies of direct PG-effects as well as studies on cerebral blood flow (CBF) utilizing cyclooxygenase inhibition have been presented. The following survey is focused on the direct cerebrovascular effects of PG´s.

Most investigators agree on the vasoconstrictor properties of $PGF_{2\alpha}$, TXA_2 and stable TX-agonists (Ellis et al. 1977, Dutton et al. 1981, Paul et al. 1982; for review, see Pickard 1981, White & Hagen 1982). In most studies PGI_2 has been found to induce cerebral vasodilation and to increase the CBF (for review, see Pickard 1981, White & Hagen 1982) but there are also reports demonstrating

that in some extracranial vascular preparations it is mainly a vasoconstrictor (Dusting et al. 1977, Levy 1978).

In contrast to the consent on the effects of $PGF_{2\alpha}$, TXA_2, and to some extent PGI_2, the findings with A-, D- and E- PG´s are conflicting (for review, see Pickard 1981, White & Hagen 1982), i.e. these PG´s have been described both as potent vasoconstrictors and vasodilators. Such divergent results may reflect not only differing experimental conditions (Pickard 1981, Uski et al. 1981, White & Hagen 1982), but also species differences (Pickard 1981, White & Hagen 1982). The results with LT´s are similarly discrepant. Some state that LTC_4 and LTD_4 are potent cerebral vasoconstrictors (Beckett & Boullin 1981, Aitken et al. 1982) while others have found these LT´s to be inactive on isolated cerebral arteries (Brandt et al. 1982, von Holst et al. 1982). Apart from results showing that B-PG´s are potent constrictors of mouse pial arterioles in vivo (Rosenblum 1975) and that PGB_2 is a constrictor of unspecified potency in the canine basilar artery (White 1979), very little is known about their cerebrovascular activity.

Since PGI_2 has a half-life of about only 3 min in aqueous solution at neutral pH (Moncada & Vane 1978), stable metabolites may contribute to the activity ascribed to PGI_2. At least one of these metabolites, 6-keto-PGE_1 (Quilley et al. 1980, Wong et al. 1980-1981), is capable of relaxing vascular smooth muscle (Coceani et al. 1980, Nandiwada et al. 1980, Quilley et al. 1980, Brandt et al. 1981b, Wong et al. 1981).

Prostaglandin receptors

Recently, attempts have been made to characterize the receptors mediating PG-induced effects in different tissues. In smooth muscle, receptors mediating contraction can be divided into those sensitive either to TXA_2 (TP), to $PGF_{2\alpha}$ (FP) or to E-PG´s (EP). The EP-receptor population can be further subdivided into EP_1- and EP_2-receptors, the former being blocked by the drug SC 19220, in contrast to the latter (Kennedy et al. 1982). Relaxant PG-induced responses in vascular smooth muscle can be mediated by receptors sensitive to PGI_2 and/or PGE_2 (Lumley et al. 1982, Jones et al. 1982, Town et al. 1982).

Except for a short report suggesting the presence of a TP-receptor in human BA obtained post-mortem (Forster & Whalley 1981), so far no studies on the characterization of PG-receptors in cerebral vessels seem to have emerged. Since PG´s may be involved in the normal regulation of cerebral blood flow (Pickard 1981) such studies are of great importance. Furthermore, disturbances in the PG-synthesis have been suggested to be involved in the pathogenesis of various pathological conditions such as cerebral vasospasm (Boullin & Blaso 1977, Boullin et al. 1979, Jarman et al. 1979, Brandt et al. 1981b, Sasaki et al. 1981, Quintana et al. 1982, White & Hagen 1982), migraine (White & Hagen 1982, Parantainen & Vapaatalo 1983) as well as other cerebrovascular diseases (White et al. 1983). Specific PG-receptor antagonists and/or agonists may, therefore, be of value in the treatment of these conditions.

Excitation-contraction coupling

Cytoplasmatic calcium plays a central role in mediating smooth muscle contraction. The intracellular free calcium concentration can be increased by transmembrane influx or by release from cellular calcium stores (Bolton 1979, Godfraind 1981). These stores may be intracellular (Bolton 1979, Brading 1981), but calcium can also be stored in the cell membrane; in the lipid bilayer of the membrane (Pang 1980, Brading 1981), in the amorphous glycocalyx coating of the lipid bilayer (Langer et al. 1982) or in membrane vesicles (Gabella 1971-73, McGuffee & Bagby 1976). Through a narrow passage, the interior of the membrane vesicles communicates with the extracellular medium, but the glycocalyx probably covers the openings of the vesicles (Gabella 1973, Langer et al. 1982). Calcium originating from the extracellular medium, glycocalyx, or membrane vesicles must pass through the lipid bilayer of the cell membrane in order to reach the cytoplasm (Fig 2).

Calcium can pass through the cell membrane via different pathways. During resting condition there is a slow passive exchange of calcium through the membrane (Weiss 1981, van Breemen et al. 1982, Loutzenhiser & van Breemen 1983). This leads to a small net calcium-influx, which is counterbalanced by an active

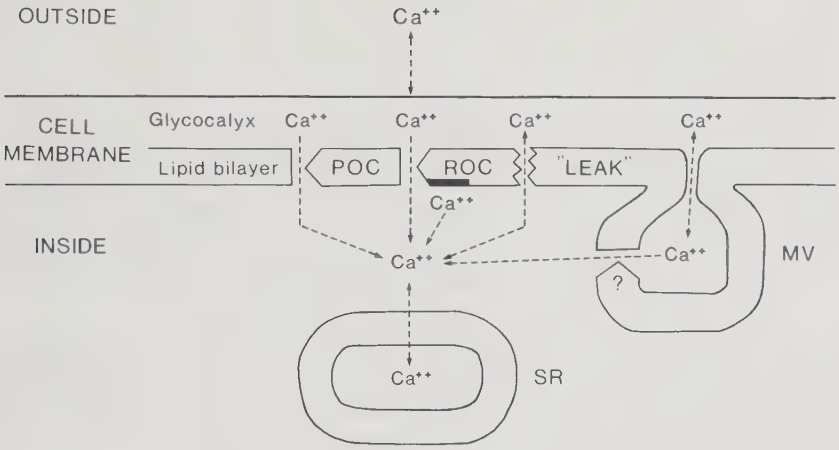

Fig 2: Schematic (and highly simplified) drawing of the possible origins of free cytoplasmatic calcium (Ca^{++}) mediating smooth muscle contraction. Calcium may enter from the extracellular medium, either from the extracellular fluid or after release from stores in the glycocalyx and membrane vesicles (MV). Channels permitting transmembrane calcium-influx may be potential operated (POC) or receptor operated (ROC). In addition, a passive leak permitting slow exchange between the extra-and intra-cellular media is illustrated. The cytoplasmatic calcium concentration may also be increased by a release from intracellular stores , such as the sarcoplasmatic reticulum (SR), mitochondria and the interior of the cell membrane.

extrusion mechanism, and does not directly participate in the excitation-contraction coupling (EC-coupling) process (Brading 1981, Langer et al. 1982, Loutzenhiser & van Breemen 1983). During activation ("excitation") specific membrane-channels, from the heart known as "slow channels" (Fleckenstein 1977, Bolton 1979) are opened. It is generally believed that there are two types of calcium channels in smooth muscle, i.e. receptor operated channels (ROC´s), opened by a receptor-agonist interaction and potential operated channels (POC´s), opened by a depolarization of the cell membrane (Bolton, 1979) (Fig 2).

There is evidence that cerebrovascular smooth muscle contraction is mainly dependent on influx of calcium from the extracellular space, whereas extracranial arteries utilize intracellularly stored calcium to a greater extent. This may explain the cerebrovascular preference for calcium channel blocking drugs, for instance substances of the dihydropyridine group such as nifedipine and nimodipine (Triggle 1981, Andersson at al. 1983, Cauvin et al. 1983). An alternative explanation is that ROC´s in cerebral arteries are more susceptible to calcium antagonists than those in peripheral vessels (Cauvin et al. 1983, Kazda & Towart 1982). Consequently, nimodipine has recently been suggested for prevention of cerebral vasospasm after aneurysmal subarachnoid hemorrhage (Allen & Bahr 1979, Brandt et al. 1979, Edvinsson et al. 1979, Auer et al. 1982, Allen et al. 1983, Ljunggren et al. 1984 a and b).

Prostaglandins and excitation-contraction coupling

Several investigators have claimed that PG-induced smooth muscle contraction is mediated by release of calcium from intracellular stores (McNamara et al. 1980, Wheeler & Weiss 1980, Loutzenhiser & van Breemen 1981), while others have found PG-induced contractions to be highly dependent on calcium in the extracellular medium (Smith et al. 1981, Godfraind & Miller 1982).

In cerebral arteries, nifedipine effectively counteracts contractions elicited by $PGF_{2\alpha}$ (Brandt et al. 1981a, Andersson et al. 1983), indicating that PG-induced activation of the contractile proteins in these vessels is mainly dependent on calcium-

influx through the cell membrane, even if contractions in human pial arteries induced by potassium seem to be more susceptible to calcium channel blockade (Brandt et al. 1981). However, Toda and co-workers (1982) have reported that contractions induced by a stable TXA_2-analogue in the canine basilar artery can not be markedly attenuated even after prolonged pretreatment in calcium-free medium, suggesting that these contractions are relatively independent of extracellular calcium. This finding is contradictory to the opinion that contractions in cerebral arteries are highly dependent on transmembrane influx of calcium, and if one does not postulate that nifedipine has effects other than calcium entry blockade or that $PGF_{2\alpha}$ and TXA_2 acts on different receptors, it is also apparently in conflict with the nifedipine-reports (see above). The mechanisms of prostanoid-induced contractions in cerebral arteries do not seem to be established.

AIMS

The present study was performed in order to investigate the effects of prostanoids on cerebral arteries with the specific aims to:

1) Compare the direct contractile and relaxant effects of a variety of prostanoids on human and feline cerebral vessels.

2) Explore the nature of the prostanoid receptors mediating contraction and relaxation in human and feline cerebral arteries.

3) Study some aspects on the mechanisms coupling prostanoid receptor stimulation with cerebrovascular smooth muscle activation in order to elucidate the following questions:

a) To what extent are $PGF_{2\alpha}$-induced contractions dependent on free extracellular and cellularly bound calcium?
b) What is the nature of the calcium channel opened by $PGF_{2\alpha}$?
c) What are the relative contributions of intracellularly- and membrane-bound calcium to $PGF_{2\alpha}$-induced contractions?

METHODS

Animal arteries

After cats were exsanguinated under sodium pentobarbital anaesthesia, the brains were removed. The cortical, middle cerebral (MCA) and basilar (BA) arteries were dissected out and used immediately or stored in Krebs solution at 4° C (for composition, see below) for up to 24 h.

Human pial arteries

Specimens of human pial arteries were obtained from macroscopically normal parts of the cortex before lobe-resections were performed in patients with deeper located gliomas. The arteries were dissected free from surrounding tissues and treated as described above.

Recording of mechanical activity

Ring segments of the arteries were suspended between L-shaped metal prongs in water-mantled organ baths containing 5 ml of Krebs solution which was kept at 37° C (Fig 3). In some experiments TRIS- or HEPES-buffered solutions were used (for compositions; see below). The Krebs solution was bubbled with 5 % CO_2 in O_2 and the other solutions were oxygenated with 100 % O_2. One of the two prongs was connected to a displacement device, whereas the other prong was connected to a force transducer registrating isometric tension. The output was recorded on an inkwriting polygraph.

Solutions

The following solutions were used:

1) "Normal Krebs" solution. This contained (in mM); NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11.0.

2) Depolarizing solutions containing 124 mM potassium (K^+). These were obtained by exchanging NaCl with equimolar amounts of KCl.

3) Calcium-free solutions. These were obtained by omitting $CaCl_2$ and adding 10^{-5} M EGTA (ethyleneglycol bis (β-aminoethylether)-N,N,N´,N´,-tetra-acetic acid).

4) TRIS or HEPES-buffered solutions. These were obtained by omitting $NaHCO_3$ and NaH_2PO_4, and adding either of 5 mM TRIS

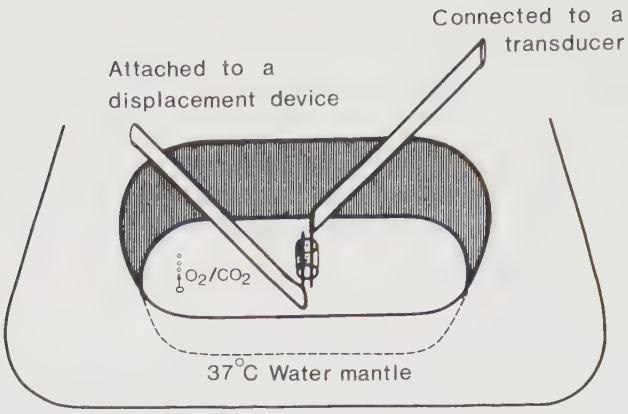

Fig 3: Schematic drawing of the central parts of the organ bath.

(tris(hydroxymethyl)-aminomethan) or 2.5 mM HEPES (N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid) and NaCl to maintain osmolality. The pH was adjusted by addition of either HCl or NaOH.

Drugs

The following drugs were used:

Prostaglandins (PG´s) A_1, A_2, B_1, B_2, D_1, D_2, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, prostacyclin (PGI_2), the PG endoperoxide analogue U44069, the thromboxane (TX) A_2 agonist U46619 (Kennedy et al. 1982), TXB_2, noradrenaline (NA), 5-hydroxytryptamine (5-HT), phentolamine, the EP_1-receptor blocker SC 19220 (Kennedy et al. 1982), nifedipine, diltiazem, manganese (Mn^{2+}) chloride, sialidase (neuraminidase type X), caffeine and the dihydropyridine derivative BAY K 8644. All drug concentrations are given as the final concentrations attained in the organ bath.

Experimental procedures

The following procedure was used to estimate potency and intrinsic activity. Reproducible contractions were obtained with a depolarizing solution containing 124 mM K^+ (for

composition, see above). All subsequent contractile responses were related to this contraction. Relaxant responses were studied in arteries contracted by various agents. The relaxation was expressed as % reduction of the response induced by the contractile agents. Except PGI_2-experiments (for explanation, see I), all concentration-response curves were obtained by cumulative addition of the drugs to the organ bath. The responses were characterized with regard to the maximum responses (E_m) that could be obtained, and to the drug concentrations resulting in half-maximum effect (EC_{50}). Calculations of the EC_{50}-values were based on the geometrical means.

In order to estimate the dependency on free extracellular calcium, the responses to $PGF_{2\alpha}$, noradrenaline (NA) and K^+ were studied after incubation of the various arterial segments in calcium-free Krebs solution containing 10^{-5} M EGTA (for composition, see above). When indicated, higher EGTA-concentrations were used. The contractile responses in calcium-free solution were expressed as % of the mean of two reproducible (±10 %) contractions obtained in normal Krebs solution with the agents studied. After incubation in calcium-free medium and subsequent addition of different drugs, the effects of a stepwise increase of the free extracellular calcium concentration was studied.

Statistical analysis

Statistical comparison between groups of data were performed by means of Student's two-tailed *t*-test. The 0.05 level of probability was accepted as significant. Results presented in text, tables or illustrations are expressed as mean values ± standard error of the mean (SE); n=number of experiments.

Effects of prostacyclin on feline and human cerebral arteries (I)

PGI_2 had a contractile effect in **feline** vessels at low resting tension: the basilar artery (BA) was contracted by 132 % and the middle cerebral artery (MCA) by 23 % of the K^+-induced (124 mM) contraction. In K^+-contracted feline arteries, including cortical vessels, addition of PGI_2 induced a further contraction; no relaxation occurred. When the arteries were contracted by $PGF_{2\alpha}$ (2×10^{-5} M), PGI_2 induced a relaxation which was more pronounced in the MCA than in the BA: The EC_{50}-values were $(1.2 \pm 1.3) \times 10^{-7}$ M and $(4.5 \pm 1.2) \times 10^{-7}$ M, respectively. PGI_2 consistently and profoundly relaxed the MCA contracted by the PG endoperoxide analogue U44069 (3×10^{-6} M), whereas in the BA the relaxant effect was both inconsistent and weak (Fig 4).

In **human** pial arteries at low resting tension, PGI_2 was inactive in concentrations below 10^{-6} M. At higher PGI_2-concentrations a weak contraction was induced (Fig 4). In contrast to feline cerebral vessels, PGI_2 consistently relaxed K^+-contracted (35 or 124 mM) human pial arteries at concentrations below 10^{-6} M. At higher concentrations further contractions were elicited (Fig 4). The EC_{50}-value for the relaxation in arteries contracted by 124 mM K^+ was $(2.9 \pm 1.1) \times 10^{-8}$ M. Human pial arteries, contracted either by $PGF_{2\alpha}$ (2×10^{-5} M), U44069 (3×10^{-6} M), noradrenaline (10^{-5} M) or 5-hydroxytryptamine (10^{-5} M), all relaxed in response to PGI_2 ($< 10^{-6}$ M). The EC_{50}-value for $PGF_{2\alpha}$-contracted vessels was $(3.7 \pm 1.5) \times 10^{-8}$ M.

The PGI_2-metabolite 6-keto-PGE_1 had effects similar to those of PGI_2, but was about ten times less potent in human pial arteries. However, in the feline MCA it was at least as potent as PGI_2, while it was inactive in the feline BA. 6-Keto-$PGF_{1\alpha}$, the hydrolysis product of PGI_2, was inactive in all vessels. 6,15-Diketo-$PGF_{1\alpha}$ had a weak relaxant effect on feline MCA and human pial arteries, but had no effect on feline BA.

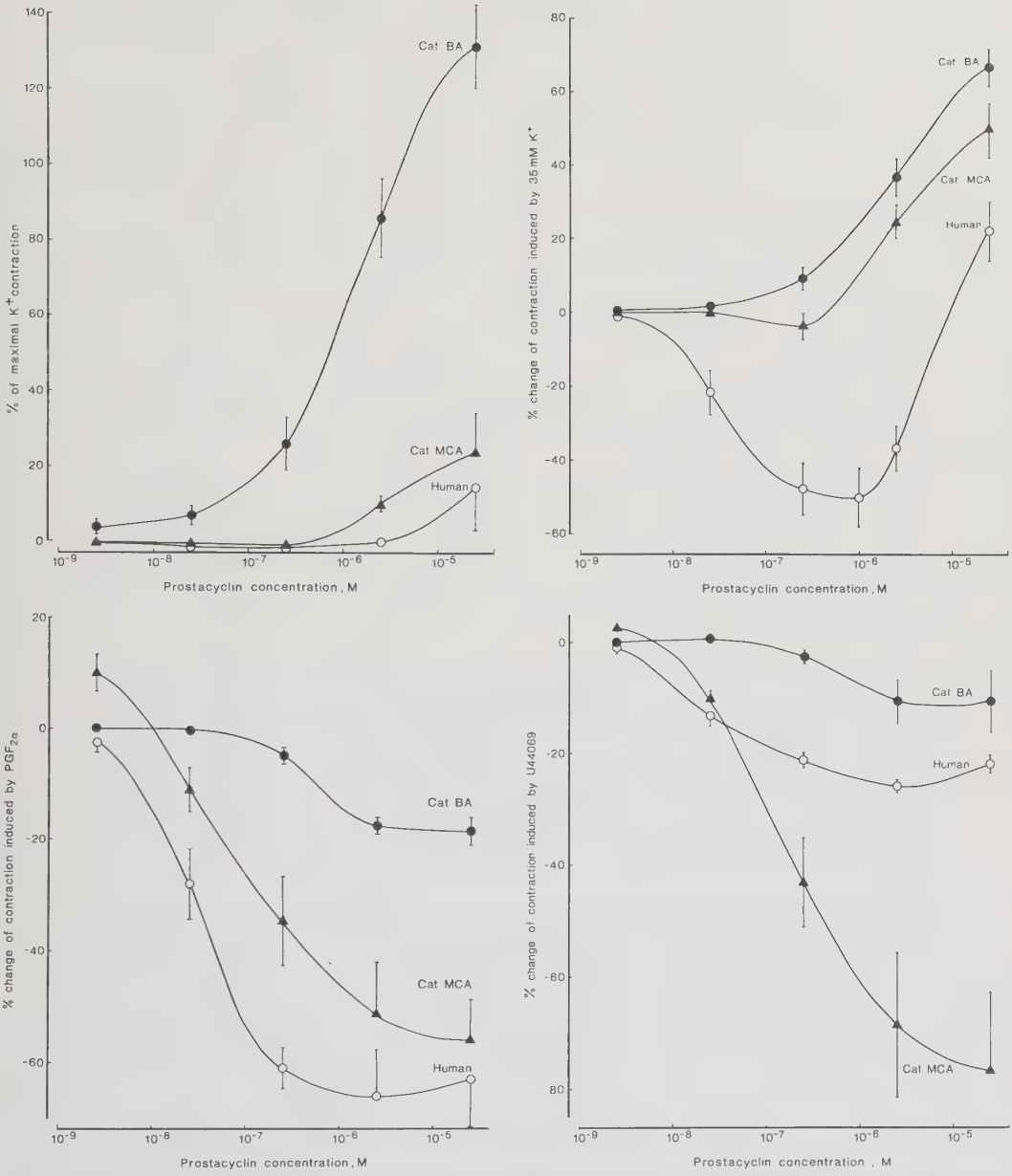

FIG. 4. Responses to PGI_2 (means ± SEM) of cat basilar artery (BA, filled circles), cat middle cerebral artery (MCA, triangles), and human pial artery (open circles). **A (top, left):** responses of vessels with low resting tension (n = 7, 5, and 3, respectively), expressed as percent of maximal potassium-induced (127 m*M*) contraction; **B (top, right):** responses of vessels contracted by 35 m*M* potassium (n = 3, 4, and 4, respectively), expressed as percent change of the contraction; **C (bottom, left):** responses of vessels contracted by $PGF_{2\alpha}$ (n = 5, 4, and 5, respectively), expressed as in B; **D (bottom, right):** responses of vessels contracted by U-44069 (n = 8, 4, and 4, respectively), expressed as in B.

Characterization of the receptors mediating prostanoid-induced cerebrovascular contraction (II, IV)

Cumulative concentration-response curves were obtained in the **feline** basilar artery (BA) with the following order of contractile potency: U46619 ≈ U44069 > PGB_2 > $PGF_{2\alpha}$ ≈ PGA_2 ≈ PGB_1 ⩾ PGA_1 ≈ PGE_2 = PGD_2 > $PGF_{1\alpha}$ ≈ TXB_2 ≈ PGE_1 ≈ PGD_1. Of particular interest are the high potencies of U46619, U44069 and PGB_2. The EC_{50}-values were (1.4 ± 1.3) x 10^{-9} M, (2.3 ± 1.2) x 10^{-9} M and (8.7 ± 1.3) x 10^{-8} M respectively. $PGF_{2\alpha}$ was less potent with an EC_{50}-value of (2.8 ± 1.6) x 10^{-7} M. PGA_1, PGA_2, PGD_1 and PGE_2 induced distinct bimodal responses with relaxation at low concentrations followed by contraction at high concentrations.

Concentration-response curves were also obtained for **human** pial arteries with a variety of prostanoids. The following order of contractile potency was obtained: U46619 ≈ U44069 > PGB_2 > $PGF_{2\alpha}$ > PGE_2 ≈ PGD_2 ≈ $PGF_{1\alpha}$ ⩾ TXB_2. U46619 and U44069 had EC_{50}-values of (7.1 ± 1.5) x 10^{-9} M and (7.9 ± 1.1) x 10^{-9} M, respectively. PGB_2 (EC_{50} = (7.0 ± 1.2) x 10^{-7} M) was also more potent than $PGF_{2\alpha}$ (EC_{50} = (2.2 ± 1.3) x 10^{-6} M). PGD_1 and PGE_1 had no contractile effects.

Incubation of the feline BA for 45 min with the PGE-receptor blocking drug SC 19220 (10 μg/ml) did not displace concentration-response curves obtained with either PGE_2, $PGF_{2\alpha}$ or U46619. Pre-treatment with the α-adrenoceptor blocker phentolamine (3 x 10^{-6} M for 15 min) had no effect on $PGF_{2\alpha}$-induced contractions. $PGF_{2\alpha}$ (2 x 10^{-5} M) further contracted arteries already contracted by 124 mM potassium or 10^{-5} M noradrenaline (NA). Potassium, but not NA, induced further contractions in vessels contracted by $PGF_{2\alpha}$.

Characterization of the receptors mediating prostanoid-induced cerebrovascular relaxation (I, II, IV)

None of the tested prostanoids relaxed K^+-contracted **feline** BA. PGE_1 and PGI_2 had obvious relaxant effects in $PGF_{2\alpha}$-contracted (2 x 10^{-5} M) BA, the latter being the most potent. The EC_{50}-values were (2.1 ± 1.4) x 10^{-6} M for PGE_1 and (4.5 ± 1.2) x 10^{-7} M for PGI_2.

The order of relaxant potency in $PGF_{2\alpha}$ (2×10^{-5} M) -contracted **human** pial arteries was: $PGI_2 > PGE_2 > PGE_1 > PGD_2 \approx PGD_1$. Human vessels contracted by 124 mM K^+ were relaxed by PGE_1 and PGI_2, the latter being the most potent. The EC_{50}-values were $(1.6 \pm 1.7) \times 10^{-7}$ M and $(2.9 \pm 1.1) \times 10^{-8}$ M, respectively.

Influence of nifedipine and calcium-free medium on $PGF_{2\alpha}$-induced contractions (III, IV)

Addition of nifedipine (3×10^{-6} M) to $PGF_{2\alpha}$ (2×10^{-5} M) or K^+ (124 mM) -contracted **feline** basilar arteries resulted in an incomplete relaxation, whereas NA (10^{-5} M) -contracted vessels were completely relaxed. $PGF_{2\alpha}$-induced contractions were markedly depressed by nifedipine-pretreatment but were sustained, whereas K^+ and NA-induced contractions were both depressed and non-sustained.

Incubation of the preparations in calcium-free medium containing 10^{-5} M EGTA for 5 - 10 min almost abolished K^+ and NA-induced contractions. In contrast, contractions induced by $PGF_{2\alpha}$ were much less affected (Table 1); 63 % of the contraction remained after 40 min pretreatment.

In **human** pial arteries, incubation in calcium-free medium for 5 - 10 min abolished K^+-induced contractions, whereas $PGF_{2\alpha}$ induced a contraction that reached 70 ± 11 % (n=10) of control also after 40 min incubation in calcium-free medium.

Table 1. *Effects of varying incubation times in calcium-free solution on contractions induced by $PGF_{2\alpha}$ (2×10^{-5} M), noradrenaline (NA, 10^{-5} M) or K^+ (124 mM)*

The responses are expressed as % of the contraction induced in calcium-containing solution. *p* indicates level of probability between responses induced by $PGF_{2\alpha}$ and K^+ or NA respectively (Student's *t*-test)

	Time in calcium-free medium							
	1 min	n	5 min	n	10 min	n	40 min	n
K^+	29±6	5	10±6	5	5±2	5	3±1	5
p	<0.01		<0.001		<0.001		<0.001	
$PGF_{2\alpha}$	70±7	6	65±8	5	67±12	5	65±7	20
p	<0.001		<0.001		<0.001		<0.001	
NA	9±2	5	8±4	5	0.5±0.2	5	1±0.5	5

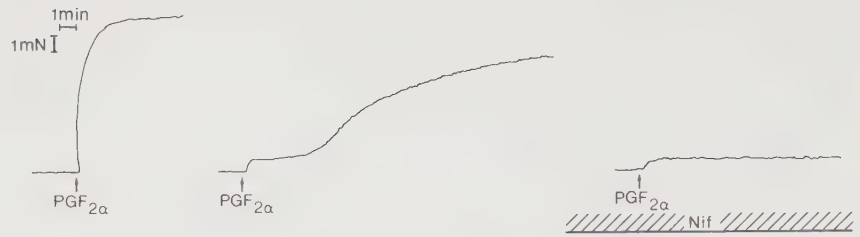

Fig 5: Tracings demonstrating the effect of 3×10^{-6} M nifedipine (NIF) on the response to $PGF_{2\alpha}$ in the feline BA pretreated with calcium-free solution. The first tracing is obtained in calcium-containing solution whereas the succeeding responses are obtained in the same preparation pretreated in calcium-free medium containing 10^{-5} M EGTA for at least 40 min.

The responses to $PGF_{2\alpha}$ were biphasic in both feline and human preparations after pretreatment in calcium-free medium containing 10^{-5} M EGTA for 40 min: a rapid initial contraction of low amplitude was followed by a second, slowly developing, and more pronounced increase in tension (Fig 5). This contractile pattern could be reproduced 2-4 times without intervening exposures to calcium. The second phase was absent if the arteries were pretreated with 3×10^{-6} M nifedipine for 15 min (Figs 5 and 6) or if the EGTA-concentration was increased to 10^{-4} M (Fig 6). Arteries from both species behaved similarly.

After incubation of the arteries in a calcium-free medium for 40 - 120 min and subsequent K^+-depolarization, re-addition of calcium elicited contractions in both human and feline vessels at lower concentrations in the presence of $PGF_{2\alpha}$ (2×10^{-5} M) than in controls. Nifedipine (3×10^{-6} M) almost abolished calcium-induced contractions in depolarized feline vessels, an effect which was partially overcome if $PGF_{2\alpha}$ was also present in the organ bath.

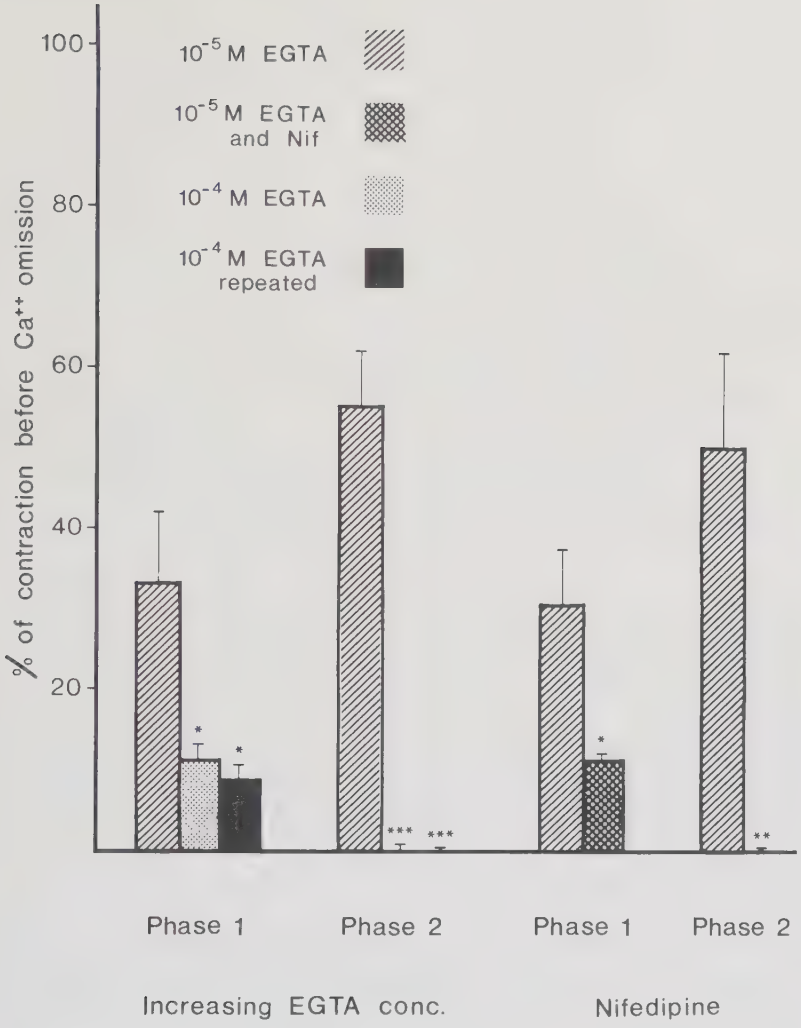

Fig 6: Effects of increasing the EGTA-concentration or pre-treatment with nifedipine (Nif) on the biphasic responses to $PGF_{2\alpha}$ in human pial arteries incubated in calcium-free medium. The contractions are expressed as % of the contraction obtained with $PGF_{2\alpha}$ in calcium-containing solution. Phase 2 was measured as the increase in tension from phase 1. n=5 in all experiments. Results significantly different from control-contractions in calcium-free medium with 10^{-5} M EGTA ($p < 0.05$, 0.01 or 0.001) are indicated by * , ** or *** respectively.

Table 2

Effects of pretreatment with various agents on the $PGF_{2\alpha}$-induced contraction in calcium-free medium.

Agent	Control Phase 1	Control phase 2	n	Pretreatment Phase 1	Pretreatment phase 2	n
Manganese (1 mM)	27 ± 4	77 ± 5	6	39 ± 3	2 ± 1 [b]	6
Diltiazem (10^{-5} M)	23 ± 4	70 ± 4	5	7 ± 1 [a]	≈0 [b]	5
Sialidase (0.5 units)	22 ± 4	71 ± 9	6	25 ± 1	29 ± 16	6
Sialidase (1.0 units)	20 ± 3	69 ± 11	5	24 ± 2	6 ± 11 [a]	5
Brief exposure to caffeine (20 mM)	25 ± 4	67 ± 8	6	11 ± 1 [a]	54 ± 12	6

Contractions are expressed as % of the contraction induced by $PGF_{2\alpha}$ in calcium-containing Krebs solution. Phase 2 was measured as the increase in tension from phase 1. Statistically significant results ($p < 0.01$ and 0.001) are indicated by [a] and [b], respectively

Further dissociation of the biphasic contraction induced by $PGF_{2\alpha}$ in calcium-free medium (V)

Feline basilar arteries (BA) pretreated in calcium-free medium for 20 min contracted 87 ± 4 % of the contraction in calcium-containing solution (n=42) in response to 2×10^{-5} M $PGF_{2\alpha}$. The responses were biphasic in all but one vessel segment. These contractions were used as controls for contractions obtained after different manipulations in calcium-free media (see below).

Manganese (Mn^{2+}) was used as an inorganic calcium channel blocker (for reasons, see V). Pretreatment with Mn^{2+} (1 mM) eliminated the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free solution, whereas the first phase was not affected (Table 2). Nifedipine (3×10^{-6} M) did not consistently relax arteries contracted by $PGF_{2\alpha}$ during exposure to Mn^{2+}. Diltiazem-pretreatment (10^{-5} M) abolished the second phase and significantly attenuated the first (Table 2).

Incubation with sialidase (1 unit/ml for 30 min), followed by a brief exposure to calcium and a subsequent 5 min period in calcium-free medium, eliminated the second $PGF_{2\alpha}$-induced phase in 4 out of 5 experiments (Table 2). Exposure to 20 mM caffeine, followed by elimination of the drug, attenuated the first phase but did not significantly affect the second (Table 2).

Effects of the "calcium promotor" BAY K 8644 on the feline BA. Influence on $PGF_{2\alpha}$-induced contractions (VI)

The recently synthetized dihydropyridine-derivative BAY K 8644 induced concentration-dependent contractions in feline basilar arteries partially depolarized with 10 mM K^+; the EC_{50}-value was $(2.1 \pm 1.2) \times 10^{-9}$ M. This calcium promotor also significantly ($p < 0.001$) displaced the concentration-response curve obtained with $PGF_{2\alpha}$ to the left.

After incubation in calcium-free medium, $PGF_{2\alpha}$ induced a biphasic response as described above. The second phase of the

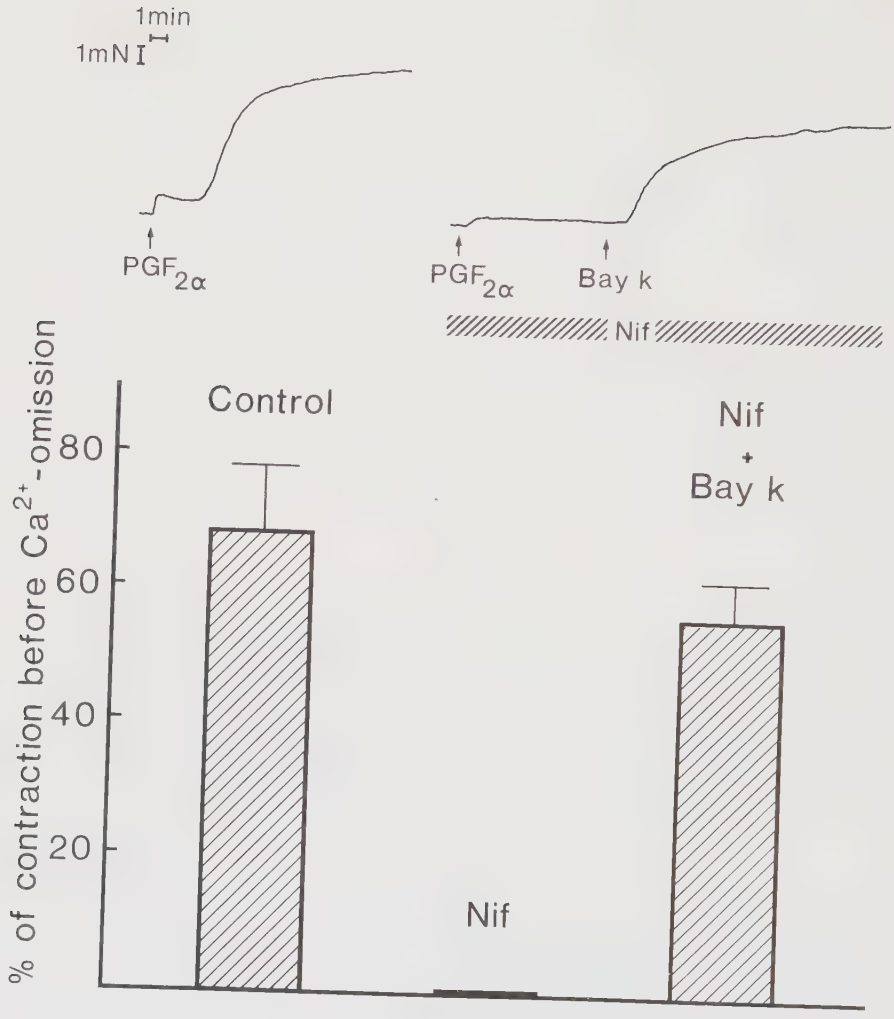

Fig 7: Effects of nifedipine (10^{-6} M), and a combination of nifedipine (Nif) and the calcium-promotor BAY K 8644 (3×10^{-6} M) on the amplitude of the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium (n=5). The contractions are expressed as % of the contraction obtained with $PGF_{2\alpha}$ in calcium-containing solution. The inset shows a representative tracing, demonstrating the effects on nifedipine and BAY K 8644 on the contractile response obtained with $PGF_{2\alpha}$ in calcium-free medium.

contraction was abolished by pretreatment with either of 10^{-6} M nifedipine or 10^{-5} M diltiazem. Addition of BAY K 8644 (3×10^{-6} M) restored the second phase in arteries incubated with nifedipine (Fig 7), but not in arteries pretreated with diltiazem.

DISCUSSION

Only scanty reports are available concerning the cerebrovascular activity of some prostaglandins such as PGB_1 and PGB_2, and for other prostanoids such as the D and E-PG´s the results are conflicting (White & Hagen 1982). So far, there seem to be no systematic studies concerning species variations in cerebrovascular responsiveness to various prostanoids.

The contractile effects on feline (II) and human (IV) brain arteries obtained by U46619, U 44069, $PGF_{2\alpha}$ and PGE_2 are well in accordance with previously published results from both peripheral (Malik & McGiff 1976, Jones et al. 1982, Kennedy et al.1982) and cerebral (Dutton et al. 1981, Pickard 1981, Paul et al. 1982, White & Hagen 1982) vessels. However, the potent vasoconstrictor activity of PGB_2 in human and in feline cerebral arteries has not previously been described, but this PG has been demonstrated to constrict mouse pial arterioles *in vivo* (Rosenblum 1975). In fact, PGB_2 was found to be more potent than the classic vasoconstrictor $PGF_{2\alpha}$ in both human (IV) and feline (II) cerebral arteries.

Prostacyclin (PGI_2) consistently relaxed K^+-contracted human pial arteries, but not corresponding feline arteries under identical conditions (I). Feline cerebral arteries pre-contracted by noradrenaline or 5-hydroxytryptamine only inconsistently relaxed in response to PGI_2, whereas human arteries were consistently and totally relaxed. However, PGI_2 had a relaxant effect in all arteries tested if these were pre-contracted by $PGF_{2\alpha}$. This demonstrates that the relaxant effect of PGI_2 is highly dependent on the agent used to pre-contract the arteries. Similar results were also observed using the A, D and E-PG´s. The existence of species differences are further supported by results showing that $PGF_{2\alpha}$ had potent contractile effects in cerebral arteries from cat (II), guinea-pig, rat (Uski, unpublished results) and man (IV), but had almost no such effect in the rabbit BA (Uski, unpublished results). The poor reactivity of rabbit cerebral arteries to PG´s has also been described by others (Hardebo et al. 1983).

There were also indications of regional differences in response to PGI_2: whereas PGI_2 consistently relaxed the feline MCA contra-

cted by U44069, the effect in the BA was small and inconsistent (I). In fact, during resting conditions, the feline BA was quite sensitive to the vasoconstrictor effect of PGI_2. Similar PGI_2-induced contractile effects have not previously been reported in cerebral vessels, except at high PGI_2-concentrations (Jarman et al. 1979, Chapleau & White 1979, Chapleau et al. 1979, Brandt et al. 1981b, Paul et al. 1982, Asano et al. 1982), but have been described in extracranial preparations in vitro (Dusting et al. 1977, Levy 1978).

PGI_2 is chemically unstable (Moncada & Vane 1978) and it is possible that active, stable metabolites may actually contribute to the effects ascribed to PGI_2. One metabolite with known vasodilator capacity, 6-keto-PGE_1 (Coceani et al. 1980, Nandiwada et al. 1980, Quilley et al. 1980, Brandt et al. 1981b, Wong et al. 1981), had effects similar to those of PGI_2 but was more than 10 times less potent on human pial arteries. All other three PGI_2-metabolites tested were inactive in both feline and human cerebral arteries (I). Therefore, it seems unlikely that any of these metabolites contributes to the effects of PGI_2 in the human cerebrovascular bed.

Both in human (IV) and feline (II) cerebral arteries the order of contractile potency was: U46619 > PGB_2 > $PGF_{2\alpha}$ > PGE_2. This is in accordance with the characteristics of the TXA_2-sensitive (TP) receptor mediating contraction proposed by Kennedy et al. (1982). The order of relaxant potency in K^+-contracted human pial arteries was: PGI_2 > PGE_1. All other tested PG´s were without relaxant effect (IV). K^+-contracted feline BA could not be relaxed by any PG (II), whereas the BA was markedly relaxed by both PGE_1 and PGI_2 if pre-contracted by $PGF_{2\alpha}$. As judged by the relative order of potencies, PG-induced feline (I, II) and human (I, IV) cerebrovascular relaxation seems to be mediated via a receptor similar to the relaxation-mediating PGI_2-sensitive receptor described in other vascular preparations (Lumley et al. 1982, Town et al. 1982). The proposed possibility of a co-existence of PGI_2- and PGE-sensitive receptors (Lumley et al. 1982) can, however, not be excluded.

Contractions induced by $PGF_{2\alpha}$ in the feline BA, being unaffec-

ted by phentolamine, do not appear to be mediated by release of noradrenaline from perivascular nerves (II). In fact, it seems as if PG-induced contractions are mediated by mechanisms at least partially different from those elicited with noradrenaline and K^+ (II). Even if nifedipine markedly counteracted contractions induced by these agents, clear differences were observed. After nifedipine-pretreatement, responses elicited by $PGF_{2\alpha}$ were markedly depressed but sustained, whereas contractions induced by K^+ or noradrenaline were both depressed and non-sustained (III).

Exclusion of free extracellular calcium had little effect on $PGF_{2\alpha}$-induced contractions in feline (III) as well as in human (IV) cerebral arteries. Also after 40 min incubation in calcium-free medium containing 10^{-5} M EGTA, more than 60 % of the response remained as compared to controls in vessels from both species. In contrast, 5-10 min pretreatment almost abolished contractions elicited by K^+ (feline and human vessels) and noradrenaline (feline vessels). It is obvious that PG-induced contractions are relatively independent on calcium in the extracellular medium and seem to be mediated mainly by the release of cellularly bound calcium.

Re-addition of calcium to depolarized arteries in calcium-free medium resulted in contractions. When $PGF_{2\alpha}$ was also present, there was a left-ward shift of the concentration-response curve to calcium in human and feline cerebral arteries (III,IV). Provided that supramaximal K^+-induced depolarization opens all potential sensitive channels (Bolton 1979), this observation indicates that $PGF_{2\alpha}$ may promote calcium influx via channels not dependent on changes in the membrane potential. Such channels, which have been called receptor operated channels (ROC´s), are believed to be less sensitive than potential sensitive channels to calcium entry blockers such as nifedipine (Bolton 1979, Vanhoutte 1982). The concentration-response curve to calcium in depolarized vessels pretreated with calcium-free solution was attenuated by nifedipine in the feline BA (III). This attenuation was partially overcome if $PGF_{2\alpha}$ was also present in the organ bath, an observation suggesting that $PGF_{2\alpha}$ may open ROC´s.

The observations that contractions induced by $PGF_{2\alpha}$ were relatively resistant to exclusion of extracellular calcium on one

hand, and that nifedipine was able to profoundly relax arteries contracted by $PGF_{2\alpha}$ on the other, are puzzling. A possible explanation is that nifedipine, in addition to calcium channel blockade, may have intracellular effects (Mikkelsen & Andersson 1978, Church & Zsotér 1980). It has, however, recently been demonstrated that nifedipine, as well as some other dihydropyridines, bind to the cell membrane, presumably at the calcium channels (Glossmann et al. 1982). This, as well as other findings, suggest that their main site of action is in the membrane, inhibiting calcium-influx to the cell (Vanhoutte 1982, Cauvin et al. 1983, Lee & Tsien 1983, Andersson & Högestätt 1984). A more attractive explanation to the conflicting observations with nifedipine and calcium-free medium is that, even if $PGF_{2\alpha}$ obviously releases cellularly bound calcium, this mediator-calcium must pass through the cell membrane, which implies that $PGF_{2\alpha}$ releases calcium bound to the outside of the cell membrane. These possible binding sites may be membrane vesicles (Gabella 1971-73, McGuffee & Bagby 1976), binding sites in the lipid bilayer (Pang 1980, Brading 1981) or sialic acid in the glycocalyx layer of the cell membrane (Jaques et al. 1977, Langer et al. 1982).

In calcium-free medium, the contraction elicited by $PGF_{2\alpha}$ was biphasic. The second and major phase could be eliminated by nifedipine-pretreatment or by a tenfold increase of the EGTA-concentration in the extracellular medium (III,IV). The latter of these observations suggests that $PGF_{2\alpha}$ releases superficially stored calcium. It seems, in fact,as if incubation in calcium-free medium containing 10^{-3} M EGTA is necessary for removal of superficially stored intracellular calcium (Saida & van Breemen 1983). Glossman and co-authors (1982) have recently suggested that the site of action of nifedipine is at the "outer mouths" of the calcium channels. The present observations, thus, hint at the possibility that $PGF_{2\alpha}$ may release calcium bound to the outer cell surface.

The enzyme sialidase (or neuraminidase) destroys the negatively charged sialic acid sites in the glycocalyx layer of the cell membrane (Langer et al. 1982, Rice & Webb 1984), which sites are capable of binding large amounts of calcium (Jaques et al. 1977, Langer et al. 1982). In the present study, pretreatment with

sialidase abolished the second phase of the $PGF_{2\alpha}$-induced contraction in most preparations (V), suggesting that extracellularly stored calcium indeed mediates this phase.

In order to further test the hypothesis that the elimination of the second phase by nifedipine is brought about by calcium entry blockade, experiments with the organic calcium channel blocker diltiazem, which is structurally different from nifedipine, and the inorganic calcium channel blocker manganese (Steinsland et al. 1973, Kostyuk 1981, Nayler 1983, Triggle & Swamy 1983) were performed (V). Both these fundamentally different calcium entry blockers abolished the second phase. Nifedipine did not further relax $PGF_{2\alpha}$-contracted arteries which had been pretreated with manganese. Provided that manganese has no intracellular calcium-antagonistic action, this observation suggests that the intracellular effects of nifedipine are negligible in the present experimental situation. This conclusion is further supported by the observation that the other calcium entry blocker, diltiazem, also abolished the second phase.

The recently described dihydropyridine-derivative BAY K 8644 appears to promote the influx of calcium through the cell membrane (Schramm et al. 1983 a and b). This drug restored the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium, if this phase had previously been abolished by pretreatment with nifedipine, but not with diltiazem. BAY K 8644 was also found to be a potent vasoconstrictor in the partially depolarized feline BA and to potentiate contractions induced by low concentrations of $PGF_{2\alpha}$ (VI). These observations support the proposal by Schramm et al.(1983) that the two dihydropyridines nifedipine and BAY K 8644, though with opposite effects, act on the same "receptor" in the cell membrane, in contrast to diltiazem. If so, the observations also further support the view that $PGF_{2\alpha}$ releases calcium bound to the exterior aspect of the cell membrane.

There is firm evidence in favour of the view that caffeine at high concentration liberates most of the calcium stored in the sarcoplasmatic reticulum (Endo 1977, Haeusler et al. 1981, Saida 1982, Saida & van Breemen 1983). Pretreatment of the arteries with 20 mM caffeine significantly depressed the first phase of the

$PGF_{2\alpha}$-induced contraction in calcium-free medium, but did not affect the second (V). The results from experiments with caffeine, sialidase, calcium entry blockers and the calcium entry promotor BAY K 8644, are all in agreement with the assumption that a major part of the $PGF_{2\alpha}$-induced contraction in calcium-free medium is mediated by the release of calcium bound to the exterior aspect of the cell membrane. This does not exclude that a minor fraction of the contraction induced by $PGF_{2\alpha}$ in calcium-free medium may represent release of calcium from intracellular stores. The first phase, being attenuated by a previous exposure to caffeine may, in fact, be mediated by the release of intracellularly sequestered calcium.

Even if contractions in cerebral vessels induced by $PGF_{2\alpha}$ seem to be highly resistant to exclusion of calcium in the extracellular medium, this does not a priori mean that prostaglandin-induced contractions are mediated by the release of intracellularly stored calcium. On the contrary, the calcium liberated by $PGF_{2\alpha}$ apparently must pass through the cell membrane in order to reach the contractile proteins, which implies that calcium entry blockers may efficiently counteract PG-induced cerebrovascular contraction. Calcium entry blockers may therefore be of value in the treatment of diseases in the pathogenesis of which PG-induced cerebrovascular contraction plays an important role.

- Values of the relative potencies of a variety of prostaglandins were determined on feline as well as human cerebral arteries. Based on these findings, prostaglandin-induced cerebrovascular contraction in both cat and man can be interpreted as being mediated by a TXA_2-sensitive receptor (TP-receptor), whereas relaxant responses probably are mediated via a receptor sensitive to PGI_2.

- The effects of PG´s may differ between species. PGI_2 relaxed human pial arteries even if these were contracted by excess potassium. No such effects were observed in feline vessels. These were, however, relaxed by PGI_2 if contractions had been induced by e.g. $PGF_{2\alpha}$. Such species differences must be taken into consideration when results obtained from animal experiments are discussed in relation to human cerebrovascular pathophysiology.

- The effects of $PGF_{2\alpha}$ were studied in arteries exposed to calcium-free medium. From these experiments it can be concluded that contractions elicited by $PGF_{2\alpha}$ in cerebral arteries from cat and man are relatively independent of the free extracellular calcium concentration. Instead, these contractions apparently are mediated mainly by the release of cellularly bound calcium.

- By comparing the effects of calcium-readdition to vessels pre-treated in calcium-free medium and subsequently exposed to various combinations of $PGF_{2\alpha}$, potassium and nifedipine, the following conclusions could be drawn: $PGF_{2\alpha}$ is able to promote trans-membrane influx of calcium via pathways which seem at least partially different from the potential sensitive calcium channels opened by potassium. These pathways probably represent receptor operated channels (ROC´s).

- Despite the fact that $PGF_{2\alpha}$-induced contractions are relatively independent of free extracellular calcium, these can be efficiently counteracted by the calcium channel blocker nifedipine. Different methods of affecting cellular calcium (EGTA, sialidase, caffeine), as well as varying means of blocking (nifedipine, diltiazem, manganese) and enhancing (BAY K 8644) calcium entry

were employed in order to obtain information on the localization of the calcium-stores mediating PG-induced contractions. The findings from these experiments strongly support the concept that $PGF_{2\alpha}$-induced contractions are mainly mediated by the release of calcium bound to the exterior aspect of the cell membrane. This may well explain the divergent findings from experiments with calcium entry blockers and elimination of calcium from the extracellular medium.

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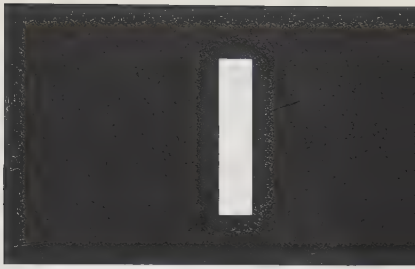

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Responses of Isolated Feline and Human Cerebral Arteries to Prostacyclin and Some of Its Metabolites

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Summary: The effects of prostacyclin (PGI_2) were studied in isolated cat basilar and middle cerebral arteries and in human pial arteries. In feline vessels with low resting tension, PGI_2 had a contractile effect that reached a maximum of 132% (basilar artery) and 23% (middle cerebral artery) of the potassium-induced (127 m*M*) contraction. In potassium-contracted feline vessels, PGI_2 caused a further contraction. When these vessels were contracted by $PGF_{2\alpha}$, PGI_2 induced relaxation, which was most marked in the middle cerebral artery. PGI_2 consistently relaxed the middle cerebral artery contracted by the prostaglandin endoperoxide analogue U-44069, whereas the basilar artery was almost unaffected. In human pial arteries with low resting tension, PGI_2 had no effects in concentrations below 10^{-6} *M*, whereas higher concentrations induced contractions. In potassium-contracted (35 or 127 m*M*) preparations, PGI_2 in concentrations below 10^{-6} *M* produced relaxation; in higher concentrations further contraction was induced. Human pial arteries contracted by $PGF_{2\alpha}$, U-44069, noradrenaline, or 5-hydroxytryptamine consistently relaxed in response to PGI_2 ($<10^{-6}$ *M*). The PGI_2 metabolite 6-keto-PGE_1 had effects similar to those of PGI_2, but proved to be less potent on human pial vessels. 6-Keto-$PGF_{1\alpha}$ was ineffective, whereas 6,15-diketo-$PGF_{1\alpha}$ had minor relaxant effects. The results suggest that consideration must be given to regional as well as species differences concerning the cerebrovascular effects of PGI_2. **Key Words:** Feline cerebral arteries—Human pial arteries—Prostacyclin—Prostacyclin metabolites—Species and regional differences.

In numerous studies, prostaglandins (PGs) have been shown to exert powerful effects on the vascular system (Malik and McGriff, 1976), including effects on isolated pial vessels and on the cerebral circulation (White, 1979; Pickard, 1981). PGs occur in the cerebrospinal fluid and are synthesized by brain tissue (Coceani and Pace-Asciak, 1976; Abdel-Halim, 1980), blood cells (Coceani and Pace-Asciak, 1976; Moncada and Vane, 1978), and pial vessels (Hagen et al., 1979; Abdel-Halim, 1980). Prostacyclin (PGI_2) is produced from PG endoperoxides by the enzyme prostacyclin synthetase. This enzyme is located mainly in blood vessels (Moncada et al., 1976*a*,*b*; 1977*a*) and found in particularly high concentrations in the intimal and subintimal layers of the vessel wall (Moncada et al., 1977*b*). There is abundant evidence that pial vessels are capable of producing PGI_2 (Abdel-Halim, 1980; Maeda et al., 1981; Sasaki et al., 1981).

Pronounced effects of PGI_2 have been demonstrated in peripheral blood vessels *in vitro* and in various peripheral vascular regions *in vivo* (Moncada and Vane, 1978; Moncada, 1982). Investigations of the effect of PGI_2 on cerebral vessels have mainly pointed at a dilatational action (Boullin et al., 1979; Chapleau and White, 1979; Ellis et al., 1979; Jarman et al., 1979; Aitken et al., 1980; Chapleau et al., 1980; Toda 1980) and an increase in cerebral blood flow (Branan et al., 1979; Pickard et al., 1980). PGI_2 is produced from the same precursor as several prostanoids with potent vasoconstrictor properties. Vascular tone may depend on a balance between vasodilatational and constrictive prostanoids (Moncada and Vane, 1978). Since intimal changes in pial vessels have been observed after experimental subarachnoid hemorrhage (SAH) (Alksne and Smith, 1977; Tanabe et al., 1978;

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Abbreviations used: BA, Basilar arteries; 5-HT, 5-hydroxytryptamine; MCA, middle cerebral arteries; NA, noradrenaline; PGI_2, prostacyclin; PG, prostaglandin; SAH, subarachnoid hemorrhage.

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Alksne and Branson, 1980) a PGI_2 deficiency may be involved in the pathogenesis of delayed cerebral vasospasm encountered in humans with aneurysmal SAH (Boullin and Blaso, 1977; Boullin et al., 1979; Jarman et al., 1979; Brandt et al., 1981; Sasaki et al., 1981; Quintana et al., 1982). In support of this view, Sasaki et al. (1981) and Maeda et al. (1981) found a reduced capacity to synthesize PGI_2 in canine basilar arteries (BAs) after experimental SAH.

PGI_2 is chemically unstable and is rapidly hydrolyzed to 6-keto-$PGF_{1\alpha}$ in aqueous solution at neutral pH (Moncada and Vane, 1978). Evidence has been presented that PGI_2 may be metabolized to 6-keto-PGE_1 (Quilley et al., 1980; Wong et al., 1980, 1981) or to 6,15-diketo-$PGF_{1\alpha}$ (Wong et al., 1978). The former is stable in aqueous solution and exerts a considerable vasodilatational effect (Coceani et al., 1980; Nandiwada et al., 1980; Quilley et al., 1980; Wong et al., 1981). Thus, it is possible that 6-keto-PGE_1 accounts for at least a part of the vasomotor activity ascribed to PGI_2.

The present study was performed in order to evaluate further the effects of PGI_2 and three PGI_2 metabolites (6-keto-$PGF_{1\alpha}$, 6-keto-PGE_1, and 6-15-diketo-$PGF_{1\alpha}$) on isolated feline and human cerebral vessels. Particular attention was given to regional and species differences, and to the possibility that the PGI_2 metabolites may contribute to the vasomotor activity ascribed to PGI_2. These factors may be of considerable importance for the understanding and interpretation of the effects of PGI_2 on the cerebral vasculature.

MATERIALS AND METHODS

Animals

Specimens of feline cerebral arteries were obtained from 18 adult cats (2.2–3.5 kg body weight), which were exsanguinated under sodium pentobarbital anaesthesia (Nembutal®, 30 mg/kg, i.p.). The brain was removed and the arteries were dissected out carefully under the microscope and then immediately immersed in cooled (+4°C) Krebs solution (for composition, see below). The middle cerebral arteries (MCAs) and BAs either were used immediately or stored in the buffer solution at +4°C for up to 24 h. In addition, small pial arteries were removed from the cortical surface and treated similarly.

Human material

Human pial vessels were removed from macroscopically normal parts of brain cortex before lobe resections were performed in nine patients with an underlying glioma. None of the patients had arterial hypertension. All patients were treated with betamethasone, in some cases phenytoin as well, and were operated under general anaesthesia (fentanyl, thiopental, and pancuronium). The vessel segments were removed together with small parts of adjacent normal cortex and immediately immersed in cooled Krebs solution. The specimens were then transported to the laboratory (arriving within 10 to 15 min). The arteries were freed carefully from surrounding tissues under the microscope and, thereafter, either were used immediately or stored as described above.

Preparation and recording of effects

Arterial segments, approximately 300–400 μm wide (outer diameter) and 2–3 mm long, were suspended between two L-shaped metal prongs in organ baths, each containing 5 ml of a Krebs solution. It has recently been demonstrated in our laboratory that cerebral arteries of a size down to approximately 100 μm (outer diameter) can be mounted with this procedure without causing any damage to the endothelium (Högestätt et al., 1982). The bath solution was continuously bubbled with 5% CO_2 in O_2, producing a pH of 7.4, and was maintained at 37°C. One of the two metal prongs was connected to a force transducer (Grass FT 03 C) for recording of isometric tension, and the other prong was fixed to a displacement device. Mechanical activity was recorded on a polygraph (Grass Model 7 B). Each vessel was given a tension of approximately 4 mN and allowed to accommodate for 90–120 min, during which time the fluid was replaced every 15 min (for further details, see Högestätt et al., 1981, 1982).

Solutions

The composition of the Krebs solution was (m*M*): NaCl, 119; $NaHCO_3$, 15; KCl, 4.6; $CaCl_2$, 1.5; NaH_2PO_4, 1.2; and glucose, 11.0. High-potassium solutions containing 35 or 127 m*M* K^+ were obtained by exchanging NaCl in the Krebs solution with equimolar amounts of KCl.

Drugs

PGI_2 sodium salt (Upjohn, U.S.A.) was dissolved in acetone and dispensed in test tubes. The acetone was rapidly evaporated under a stream of nitrogen, leaving the pure PGI_2 salt in the test tubes. This salt was then stored at −20°C and dissolved in ice-cold 0.05 *M* Tris-buffer solution (pH 10.4) immediately before use. Dilutions were made up in the same solvent, and the PGI_2 solutions were kept on ice thereafter until added to the organ baths in amounts of 25 μl. 6-keto-$PGF_{1\alpha}$, 6-keto-PGE_1, and 6,15-diketo-$PGF_{1\alpha}$ (Upjohn, U.S.A.) were dissolved in acetone, dispensed, and stored as described for PGI_2. The PGI_2 metabolites were redissolved in Tris buffer, and further dilutions were made with phosphate buffer. A stock solution of the prostaglandin endoperoxide analogue U-44069 [(15*S*)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid] (Upjohn, U.S.A.) was made up in absolute ethanol (10 mg/ml) and stored at −20°C. $PGF_{2\alpha}$ was supplied as an aqueous solution (Amoglandin®; Astra, Sweden). Fresh dilutions were made each day with phosphate buffer at neutral pH, and kept in the refrigerator until immediately prior to use. Noradrenaline (L-arterenol hydrochloride) (NA) and 5-hydroxytryptamine (5-hydroxytryptamine-creatinine sulphate) (5-HT) were obtained from Sigma. The concentrations of prostanoids are given as concentrations of the free acid in the organ bath. The concentrations of the amines are given as concentrations of free base.

Experimental procedure

To avoid gross changes in the pH of the fluid in the organ baths, PGI_2 was added in single doses (25 μl). The baths were rinsed at least twice before the next dose was added. As checked in separate experiments, the concentration of Tris buffer in the organ bath did not affect the resting vessel tension. Contracted vessels sometimes reacted with a further minute contraction after addition of Tris buffer. The PGI_2 metabolites were added cumulatively. Their solvent (phosphate buffer) did not affect resting vessel tension in human or feline preparations. However, feline MCAs that were contracted by 35 m*M* K^+ or $PGF_{2\alpha}$ were relaxed by 5 ± 0.4% and 10 ± 2% (n = 10), respectively, in response to phosphate buffer. This mean relaxation was subtracted from the response obtained by the substances tested.

The contraction obtained with the depolarizing solution containing 127 m*M* K^+ was set at 100%, and all subsequent contractile responses were related to this value. The potassium-induced (127 m*M*) contraction reached an amplitude of 15.0 ± 0.8 mN (n = 42) in the feline BA, 17.8 ± 0.8 mN (n = 39) in the MCA, and 8.4 ± 0.3 mN (n = 36) in human pial arteries. Relaxation was expressed as percent reduction of the response induced by the contractile agents used. Both $PGF_{2\alpha}$ and U-44069 produced contractions exceeding those induced by 127 m*M* K^+ in most of the vessels tested.

The responses were characterized further with regard to drug concentrations resulting in half-maximum contraction or relaxation (EC_{50}). The maximum responses (E_m) that could be obtained were also determined. Calculations of the EC_{50} values were based on the geometrial means. Results are presented as means ± SEM.

RESULTS

Effects of PGI_2 on feline cerebral arteries

Under resting conditions, PGI_2 produced a concentration-dependent contraction of the BA (Fig. 1A). The "threshold concentration" for contraction was 2.5×10^{-9} *M* (this concentration of PGI_2 consistently induced contractions ranging from 1 to 15%), the EC_{50} value was 1.4×10^{-6} *M* ($-\log EC_{50} = 5.9 \pm 0.1$), and the E_m value was 132 ± 11% of the potassium-induced contraction (n = 7). Pretreatment with the cyclooxygenase inhibitor indomethacin (10^{-6} *M*) did not change this reaction pattern. Contraction by PGI_2 (2.5×10^{-5} *M*) reached 94 ± 9% after indomethacin pretreatment, as compared to 91 ± 7% in controls (n = 4). Compared to the BA, the MCA was less sensitive to the PGI_2-induced contraction. In the MCA, the contraction was sometimes preceded by a weak relaxation appearing at about 10^{-7} *M* PGI_2 (Fig. 1A). In the highest PGI_2 concentration used (2.5×10^{-5} *M*), the contraction of the MCA amounted to 23 ± 11% of the potassium-evoked response (n = 5).

In other experiments, the vessels were contracted by either 35 or 127 m*M* K^+, U-44069 (3×10^{-6} *M*), or $PGF_{2\alpha}$ (2×10^{-5} *M*). In preparations contracted by 35 m*M* K^+, PGI_2 caused further contraction of the BA (Fig. 1B). PGI_2 relaxed four of eight preparations when contractions had been produced by U-44069. No change was observed in the remaining four preparations (Fig. 1D). The overall relaxant E_m value was 10 ± 4% (n = 8). A consistent but moderate (about 18%) relaxant effect was observed in $PGF_{2\alpha}$-contracted preparations (Fig. 1C; Table 1).

In the MCA contracted by 35 m*M* K^+, PGI_2 in concentrations up to 10^{-7} *M* had no consistent effects (Fig. 1B). At higher concentrations of PGI_2, contractions were seen regularly. PGI_2 did not relax either the MCA or small pial arteries contracted by 35 or 127 m*M* K^+. In the MCA contracted by 2×10^{-5} *M* $PGF_{2\alpha}$ or 3×10^{-6} *M* U-44069, a concentration-dependent relaxation was consistently obtained (Figs. 1C,D; Table 1). Pretreatment with indomethacin (10^{-6} *M*) did not affect this pattern of reaction. In $PGF_{2\alpha}$-contracted vessels, PGI_2 (2.5×10^{-6} *M*) caused a relaxation of 55 ± 12% after indomethacin pretreatment and of 49 ± 5% in controls (n = 4). In arteries contracted by NA (10^{-4} *M*) or 5-HT (10^{-5} *M*), PGI_2 up to 5×10^{-7} *M* had no consistent relaxant effect (Fig. 2). In higher concentrations PGI_2 induced further contractions.

Effects of PGI_2 on human pial arteries

PGI_2 in low concentrations ($<10^{-6}$ *M*) had no consistent effect when added to pial vessels under resting conditions. In concentrations above 10^{-6} *M*, PGI_2 had a contractile effect (Fig. 1A).

In potassium-contracted preparations, PGI_2 had a maximum relaxant effect at a concentration of 10^{-6} *M* (Fig. 1B). The E_m value for the relaxant effect was 50 ± 8% of the potassium-induced (35 m*M*) contraction and the EC_{50} value was 4.3×10^{-8} *M* ($-\log EC_{50} = 7.4 \pm 0.11$; n = 4). When contractions were produced by 127 m*M* K^+, the maximum relaxation obtained was 18 ± 3%, and the EC_{50} value was 2.9×10^{-8} *M* ($-\log EC_{50} = 7.5 \pm 0.06$; n = 3). PGI_2 produced contractions at lower concentrations in vessels contracted by 127 m*M* K^+ than in vessels contracted by 35 m*M* K^+.

$PGF_{2\alpha}$-contracted arteries relaxed in response to PGI_2 (Fig. 1C; Table 1). If contraction was induced by U-44069, the relaxation was less pronounced ($E_m = 26 \pm 1\%$, n = 4; Fig. 1D). PGI_2 ($<10^{-6}$ *M*) consistently relaxed human vessels contracted by either NA or 5-HT (Fig. 2). This relaxation sometimes reached values below those of the initial resting tension. When PGI_2 in concentrations exceeding 10^{-6} *M* was added to NA- or 5-HT-contracted vessels, further contractions were induced.

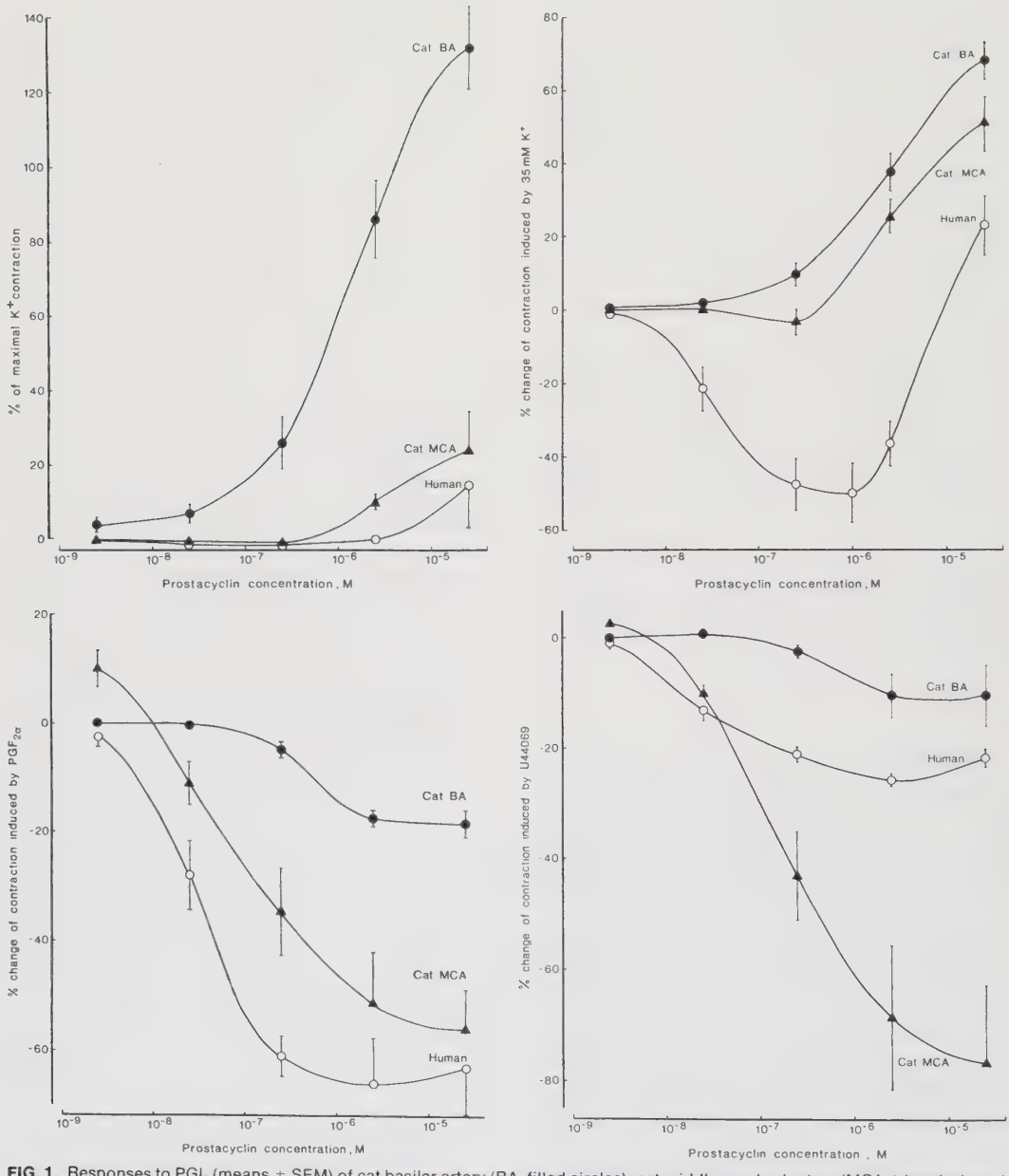

FIG. 1. Responses to PGI_2 (means ± SEM) of cat basilar artery (BA, filled circles), cat middle cerebral artery (MCA, triangles), and human pial artery (open circles). **A (top, left):** responses of vessels with low resting tension (n = 7, 5, and 3, respectively), expressed as percent of maximal potassium-induced (127 m*M*) contraction; **B (top, right):** responses of vessels contracted by 35 m*M* potassium (n = 3, 4, and 4, respectively), expressed as percent change of the contraction; **C (bottom, left):** responses of vessels contracted by $PGF_{2\alpha}$ (n = 5, 4, and 5, respectively), expressed as in B; **D (bottom, right):** responses of vessels contracted by U-44069 (n = 8, 4, and 4, respectively), expressed as in B.

TABLE 1. *Relaxant responses to PGI_2 and some PGI_2 metabolites of feline and human pial arteries contracted by $PGF_{2\alpha}$*

	Feline basilar artery			Feline middle cerebral artery			Human pial artery		
	n	EC_{50} (*M*) ($-\log EC_{50}$ ± SEM)	E_m (%)	n	EC_{50} (*M*) ($-\log EC_{50}$ ± SEM)	E_m (%)	n	EC_{50} (*M*) ($-\log EC_{50}$ ± SEM)	E_m (%)
PGI	5	4.5×10^{-7} (6.3 ± 0.08)	18.5 ± 2.5	4	1.2×10^{-7} (6.9 ± 0.11)	56 ± 8	5	3.7×10^{-8} (7.4 ± 0.17)	66 ± 8
6-keto-PGE_1	3		No effect	5	5.7×10^{-8} (7.2 ± 0.19)	24 ± 9	5	5.7×10^{-7} (6.2 ± 0.13)	56 ± 12
6-keto-$PGF_{1\alpha}$	3		No effect	5		No effect	3		No effect
6,15-diketo-$PGF_{1\alpha}$	4		No effect	5	4.6×10^{-8} (7.3 ± 0.21)	14 ± 6.5	5	1.2×10^{-7} (6.9 ± 0.43)	7.5 ± 1.5

The responses are characterized by the EC_{50} values (and the negative log of the EC_{50} values) and the maximal relaxation (E_m), expressed as percent reduction of $PGF_{2\alpha}$-induced contraction and presented as mean values ± SEM; n, number of experiments.

Effects of PGI_2 metabolites

After potassium-induced contractions, 6-keto-$PGF_{1\alpha}$ and 6-keto-PGE_1 (3×10^{-5} *M*) contracted the resting feline BA by 54 ± 8% (n = 5) and 73 ± 22% (n = 6), respectively. 6,15-Diketo-$PGF_{1\alpha}$ (3×10^{-9} to 3×10^{-5} *M*) had no effect. In BA preparations contracted by potassium or $PGF_{2\alpha}$, none of the three PGI_2 metabolites had any relaxant effect, whereas further minor contractions were seen in response to 6-keto-$PGF_{1\alpha}$ and 6-keto-PGE_1 in K^+-contracted vessels. No effect was observed in the feline MCA under resting conditions. Minor (~6%) relaxant effects were seen in the MCAs contracted by 35 m*M* K^+ after addition of 6-keto-$PGF_{1\alpha}$, 6-keto-PGE_1, or 6,15-diketo-$PGF_{1\alpha}$. The relaxant effects were more pronounced in $PGF_{2\alpha}$-contracted MCAs (Fig. 3; Table 1).

The three PGI_2 metabolites exerted no significant effects on resting human pial arteries. 6-Keto-PGE_1 relaxed vessels contracted by 35 m*M* K^+ (69 ± 9%), and the EC_{50} value was 2.3×10^{-7} *M* ($-\log EC_{50}$ = 6.6 ± 0.06, n = 4; Fig. 4). 6-Keto-$PGF_{1\alpha}$ was without consistent effect, whereas 6,15-diketo-$PGF_{1\alpha}$ relaxed the arteries by 15 ± 5% (n = 3). Similar responses were observed in $PGF_{2\alpha}$-contracted preparations (Fig. 3; Table 1). In vessels contracted by 127 m*M* K^+, the relaxation induced by 6-keto-PGE_1 ranged from 8 to 37% (n = 5).

DISCUSSION

Most previous studies concerning the cerebrovascular effects of PGI_2 have demonstrated relaxant effects (Boullin et al., 1979, human and baboon BA *in vitro;* Chapleau and White, 1979, canine BA *in vitro;* Ellis et al., 1979, cat *in vivo;* Jarman et al., 1979, baboon *in vitro* and *in vivo;* Aitken et al., 1980, rat *in vivo;* Chapleau et al., 1980, canine BA *in vitro;* Pickard et al., 1980, baboon *in vivo;* Toda 1980, canine *in vitro*). However, there have been observations of a contractile effect of PGI_2 on peripheral blood vessels (Dusting et al., 1977, pig coronary artery *in vitro;* Levy, 1978, human saphenous, rat portal, and caval veins *in vitro*). In the present study, PGI_2 was shown to produce strong contractions of the isolated feline BA under resting conditions. In high concentrations, similar effects were observed in the isolated feline MCA

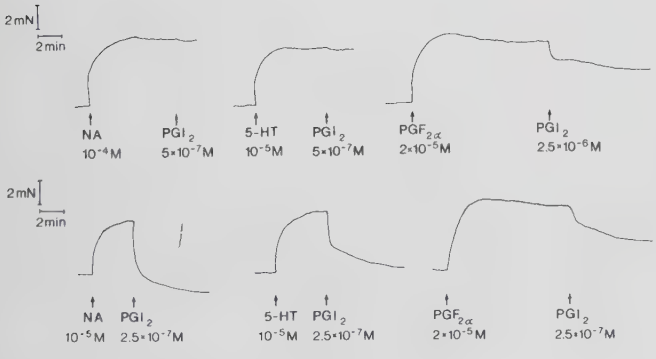

FIG. 2. Representative tracings of responses to PGI_2 in the feline middle cerebral artery **(top)** and human pial artery **(bottom)** contracted by NA, 5-HT, or $PGF_{2\alpha}$.

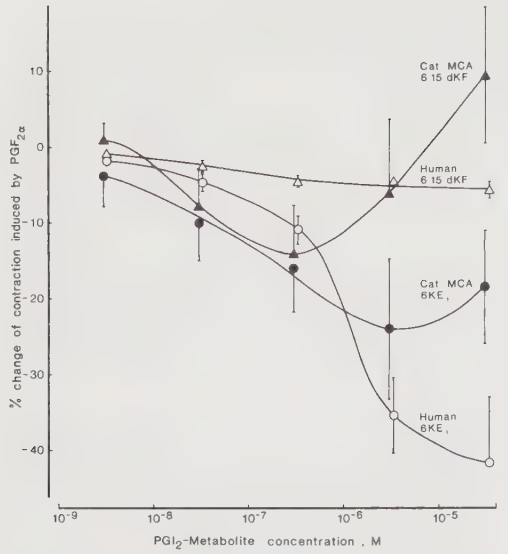

FIG. 3. Responses (means ± SEM) to 6-keto-PGE_1 ($6KE_1$) (circles) and 6,15-diketo-$PGF_{1\alpha}$ (6,15 dKF) (triangles) of vessels contracted by $PGF_{2\alpha}$. Closed symbols indicate responses of feline middle cerebral arteries (MCA) (n = 5), and open symbols, responses of human pial arteries (n = 5). The responses are expressed as percent change of the contraction induced in the vessels before addition of PGI_2 metabolites.

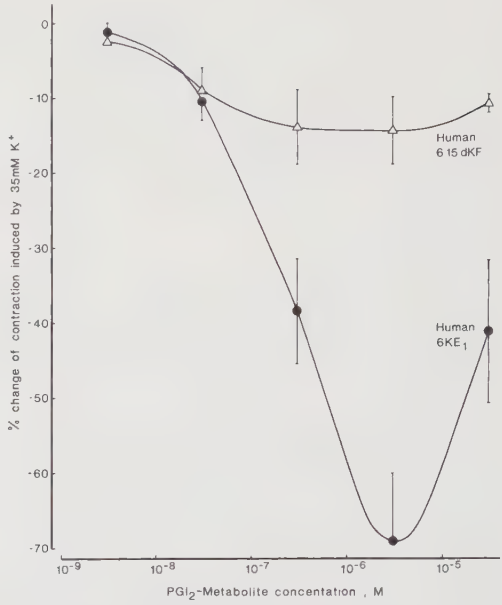

FIG. 4. Responses (means ± SEM) to 6-keto-PGE_1 ($6KE_1$, filled circles) (n = 4) and 6,15-diketo-$PGF_{1\alpha}$ (6,15 dKF; triangles) (n = 3) of human pial arteries contracted by 35 m*M* potassium. Responses are expressed as in Fig. 3.

and human cortical arteries. The present series of experiments revealed a marked difference in the response to PGI_2 between contracted feline BA and MCA. When contractions had been induced by U-44069, the MCA regularly relaxed in response to PGI_2, whereas the BA did not consistently relax. The reason for the variability in the response to PGI_2 of the BA is unclear. Also, in $PGF_{2\alpha}$-contracted vessels PGI_2 had a more marked relaxant effect on the MCA than on the BA. The discrepancies in the responses to PGI_2 between the BA and MCA may indicate quantitative regional differences in the action of PGI_2.

It is assumed that vasoconstrictor PGs contribute to the maintenance of tone in the cerebral vasculature (Pickard and MacKenzie, 1973; Ellis et al., 1979; Hagen et al., 1979; Pickard, 1981). The present results support this concept. PGs have been proposed to function as modulators of the sympathetic nervous system and as attenuators of the vasoconstrictor effects of angiotensin II (Nasjletti and Malik, 1981). In the present study, PGI_2 relaxed feline MCA contracted with prostanoid agents ($PGF_{2\alpha}$ or U-44069) consistently and to a considerable degree, but inconsistently when contracted by NA or 5-HT (PGI_2 concentrations up to 5×10^{-6} *M*). The possibility cannot be excluded that these relaxant effects were due to PGI_2 competing with $PGF_{2\alpha}$ or U-44069 for the same contracting prostanoid receptor, since PGI_2 did not produce any consistent relaxant effects on vessels contracted by potassium, NA, or 5-HT. It has previously been demonstrated that topical application of PGI_2 induces dilatation of supratentorial feline pial arterioles (Ellis et al., 1979). The possible role of endogenous "protectors" against excessive vasoconstriction has been attributed mainly to the E-prostaglandins, but PGI_2 may also have a similar role (Nasjletti and Malik, 1981). It has been suggested that PGI_2 can reduce vascular contractility by inhibiting the release of NA from adrenergic nerves (Weitzell et al., 1978; Schrör et al., 1981). On the other hand, an inability of PGI_2 to inhibit NA release in the kidney has been described (Hedqvist, 1979).

Previous studies on isolated pial arteries from humans (Brandt et al., 1981), baboons (Jarman et al., 1979), and dogs (Chapleau and White, 1979; Chapleau et al., 1980) have revealed that PGI_2 may produce a biphasic response: a relaxation at low concentrations followed by a contraction at higher concentrations. The present study confirms that

PGI_2 induces a biphasic response in isolated human pial arteries. Boullin et al. (1979) reported consistent relaxant effects of PGI_2 in human basilar arteries obtained at autopsy. However, the highest PGI_2 concentration used was 5×10^{-7} *M*. In the present study, contractions were shown to occur at PGI_2 concentrations exceeding 10^{-6} *M*.

PGI_2 is chemically unstable and has a short lifetime in aqueous solution. Therefore, active stable metabolites may contribute to the effects ascribed to PGI_2. On human pial arteries, 6-keto-PGE_1 had an effect that consistently resembled that of PGI_2. 6,15-Diketo-$PGF_{1\alpha}$ and, in particular, 6-keto-$PGF_{1\alpha}$ were much less effective. 6-Keto-PGE_1 has been reported to have a considerable dilatational activity in the peripheral circulation (Coceani et al., 1980; Nandiwada et al., 1980; Quilley et al., 1980; Wong et al., 1981). The enzyme responsible for the synthesis of 6-keto-PGE_1 has been demonstrated in the liver and in platelets (Quilley et al., 1980; Wong et al., 1980, 1981), but not as yet in brain or in cerebral vessels. The present results show that 6-keto-PGE_1 has a relaxant effect on the feline MCA and human pial arteries, but not on the feline BA. However, in human pial arteries *in vitro*, the potency was only one-tenth that of PGI_2. It therefore seems less likely that 6-keto-PGE_1 should be responsible for the main part of the effect on human cerebral vessels ascribed to PGI_2. In feline MCA, however, 6-keto-PGE_1 seems to have at least the same potency (as defined by the EC_{50} values) as PGI_2.

PGI_2 was capable of relaxing human pial arteries, even after depolarization by 35 or 127 m*M* K^+, but no such effect was found on feline vessels. This also was true for small feline cortical arteries removed from regions of the brain corresponding to the regions from which the human pial vessels used in the study were obtained. Furthermore, PGI_2 (in concentrations up to 5×10^{-6} *M*) had no consistent relaxant effect on feline MCA contracted by NA or 5-HT, in contrast to its very pronounced relaxant effect on human pial arteries contracted by these amines. The reason for these discrepancies remains obscure. One might speculate that different intracellular mechanisms mediating PGI_2-induced relaxation are involved in vessels from different species.

The data of the present study suggest important differences in vascular reactivity between human and feline cerebral vessels, as well as regional differences in the reaction of feline cerebral arteries. These facts should be taken into consideration when results obtained in animal models are used to explain the pathophysiology of human cerebrovascular disease.

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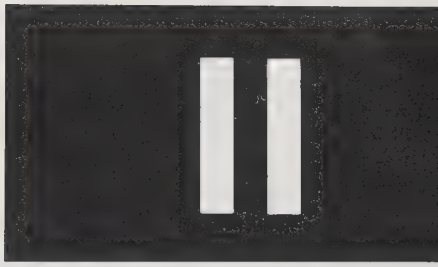

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Effects of prostanoids on isolated feline cerebral arteries

I. Characterization of the contraction-mediating receptor

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The effects of various prostanoids on isolated feline basilar arteries (BA) were studied. Contractions were induced with the following order of potency: U 46619 ≈ U44069 > PGB_2 > $PGF_{2\alpha}$ ≈ PGA_2 ≈ PGB_1 ≥ PGA_1 ≈ PGE_2 = PGD_2 > $PGF_{1\alpha}$ ≈ TXB_2 ≈ PGE_1 ≈ PGD_1. Distinct bimodal responses with a relaxation at low concentrations followed by a contraction at high concentrations, were induced by PGA_1, PGA_2, PGD_1 and PGE_2. None of the tested prostanoids relaxed K^+-contracted arteries, and a sizable relaxant effect in $PGF_{2\alpha}$-contracted arteries could be induced only by PGE_1. As judged by the relative order of potency, PG-induced contractions of the feline BA seem to be mediated by a thromboxane sensitive receptor. $PGF_{2\alpha}$-induced contractions apparently do not involve the release of noradrenaline from perivascular nerves since phentolamine failed to affect contractions induced by this agent.

Key words: Prostanoids, prostanoid receptors, contraction, isolated basilar arteries, cat

Attempts have been made to characterize different populations of prostanoid receptors in various kinds of smooth muscle. Based on studies of relative potency of prostanoids and the use of prostaglandin (PG) antagonists, at least 3 types of contraction mediating receptors have been suggested: thromboxane (TX) sensitive (TP) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) sensitive (FP), and prostaglandin E (PGE) sensitive (EP) receptors (Coleman et al. 1980*a* and *b*, Kennedy et al. 1982). PGE-sensitive receptors may be divided into two subtypes; those blocked by SC 19220, and those which are not blocked by this drug. According to the classification proposed by Kennedy et al. (1982), the former of these should be named EP_1 and the latter EP_2 receptors. Distinct contraction-mediating receptors for PGI_2 (IP) and PGD_2 (DP) seem to exist. The order of potency between different prostanoids vary between tissues depending on the type of PG-receptor present (Kennedy et al. 1982).

The present study was performed in order to compare the effects of a variety of prostanoids on the feline basilar artery (BA). The objective was to obtain information on the nature of the prostanoid receptor mediating contractions in this vessel. The possibility that noradrenaline (NA) release from perivascular nerves might contribute to PG induced contractions was also investigated.

MATERIALS AND METHODS

Preparation

Adult cats of either sex were exsanguinated under sodium pentobarbital anaesthesia (Nembutal, 30 mg/kg intraperitoneally). Immediately after sacrifice a craniotomi was performed and the brain was removed. The basilar artery (BA) was thereafter carefully dissected free from underlaying brain tissue under the microscope and promptly immersed in cooled (about 4°C) Krebs solution (for composition, see below). The arteries were either used immediately or stored in the Krebs solution at 4°C for up to 24 h.

Recording of mechanical activity

The BA was cleaned from adherent arachnoid membranes and divided into 2–3 mm long segments. These ring segments, approximately 300–400 μm wide, were suspended between two L-shaped metal prongs in 5 ml temperature controlled water mantled organ baths containing Krebs solution (for methodological details see Högestätt, Andersson & Edvinsson, 1983). The Krebs solution was maintained at 37°C and continuously gassed with a mix-

Table 1. *Contractile responses to some prostanoids characterized by their EC_{50}- and E_m-values*

The contractions are expressed as % of the contraction induced by 124 mM K^+

Agent	EC_{50} (M)	E_m (%)	n
PGA_1	$(8.4\pm2.0)\times10^{-7}$	131±24	5
PGA_2	$(3.0\pm1.4)\times10^{-7}$	135±22	5
PGB_1	$(3.8\pm1.3)\times10^{-7}$	114±20	5
PGB_2	$(8.7\pm1.3)\times10^{-8}$	109±15	6
PGD_1	$>2.7\times10^{-5}$	>20	6
PGD_2	$(1.3\pm1.2)\times10^{-6}$	132±24	6
PGE_1	$>8.0\times10^{-6}$	>110	6
PGE_2	$(1.3\pm1.2)\times10^{-6}$	147±21	8
$PGF_{1\alpha}$	$>2.4\times10^{-6}$	>104	5
$PGF_{2\alpha}$	$(2.8\pm1.6)\times10^{-7}$	117±11	7
TXB_2	$>4.3\times10^{-6}$	>100	5
U46619	$(1.4\pm1.3)\times10^{-9}$	109±4	5
U44069	$(2.3\pm1.2)\times10^{-9}$	131±13	5

ture of 95% O_2 and 5% CO_2, resulting in a pH of about 7.4. One of the two metal prongs was connected to a force transducer (Grass Model FT 03C) for registration of isometric tension and the other to a movable unit allowing fine adjustments of passive tension. The output from the transducer was recorded on a polygraph (Grass Model 7B). During an equilibration period of 60–90 min vessel tension was adjusted stepwise to a final value of 4–5 mN. The buffer solution was exchanged every 15 min.

Solutions

The following solutions were used: (1) "Normal Krebs" solution with the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11.0. (2) A depolarizing solution containing 124 mM potassium (K^+) was obtained by exchanging NaCl with equimolar amonts of KCl.

Drugs

Stock solutions (10 mg/ml) of the following prostanoids were made up in absolute ethanol: PGA_1, PGA_2, PGB_1, PGB_2, PGD_1, PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$, thromboxane B_2 (TXB_2), the prostaglandin endoperoxide analogue U44069 [(15 S)-hydro-9α, 11α-(epoxymethano)prosta-5Z, 13 E-dienoic acid] and the proposed TXA_2 agonist (Coleman et al., 1980*b*, Kennedy et al., 1982) U46619 (15 S)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13 E-dienoic acid] (Upjohn, USA). All stock solutions were stored at −20°C. $PGF_{2\alpha}$ was supplied as an aqueous solution (Amoglandin®, Astra, Sweden) and was stored at 4°C. Dilutions were made fresh each day with phosphate buffer at neutral pH, and were kept in the refrigerator until immediately prior to use. Stock solutions of SC-19220 (D. G. Searle & Co. USA) were made up in ethanol (10 mg/ml) and diluted with 0.9% NaCl. Phentolamine methane sulfonate was obtained from Ciba Geigy and was dissolved in 0.9% NaCl with 1 mM ascorbic acid (as an antioxidant) and diluted with the same solvent. The concentrations of the agents are given as the final concentrations of the free acid or base in the organ bath.

Experimental procedure

Reproducible contractions (±10%) induced by the depolarizing Krebs solution containing 124 mM K^+ were required before a preparation was accepted for further experiment. All subsequent contractile responses were expressed as per cent of the maximum K^+-induced contraction. In experiments where relaxant responses to prostaglandins were studied in contracted arteries, relaxation was expressed as per cent reduction of the response induced by the contractile agents. Concentration-response curves were constructed on the basis of results obtained by adding the prostanoids cumulatively to the organ bath. Both contractile and relaxant responses were further characterized with regard to drug concentrations resulting in half-maximum effect (EC_{50}). The maximum responses (E_m) that could be obtained were also determined. Whenever appropriate, the results presented in text, tables or figures are expressed as mean values ± standard error of the mean (SE), n number of experiments.

RESULTS

Depolarization of the vessels by 124 mM K^+ resulted in a contraction which reached a maximum tension of 12.8±0.6 mN (n=79). Pretreatment of the vessels with 3×10^{-6} M phentolamine decreased the contraction by less than 10%; 10^{-6} M atropine had no consistent effect on the response to K^+.

In the BA cumulative concentration-response-curves for different prostanoids were obtained in normal Krebs solution (Figs. 1 *a–d*). PGA_1, PGA_2, PGD_1 and PGE_2 induced a distinct bimodal response: a relaxation at low concentrations followed by a contraction at high concentrations. The other substances had only contractile effects. The following order of contractile potency was obtained: U46619 ≈ U44069 > PGB_2 > $PGF_{2\alpha}$ ≈ PGA_2 ≈ PGB_1 ≥ PGA_1 ≈ PGE_2 = PGD_2 > $PGF_{1\alpha}$ ≈ TXB_2 ≈ PGE_1 ≈ PGD_1. The EC_{50} and E_m-values are presented in Table 1. Within the concentration-range studied, the curves for $PGF_{1\alpha}$, TXB_2, PGE_1 and PGD_1 did not reach a plateau and consequently EC_{50} and E_m-values for these substances could not be determined. After contractions had been induced by U46619 or U44069, the vessels relaxed very slowly, even after repeated washings, and not infrequently failed to reach their basal tension level. Incubation of the arteries for 45 min with the proposed PGE-receptor blocking agent SC 19220 (Coleman et al. 1980, Kennedy et al. 1982) in a concentration of 10 μg/ml failed to displace concen-

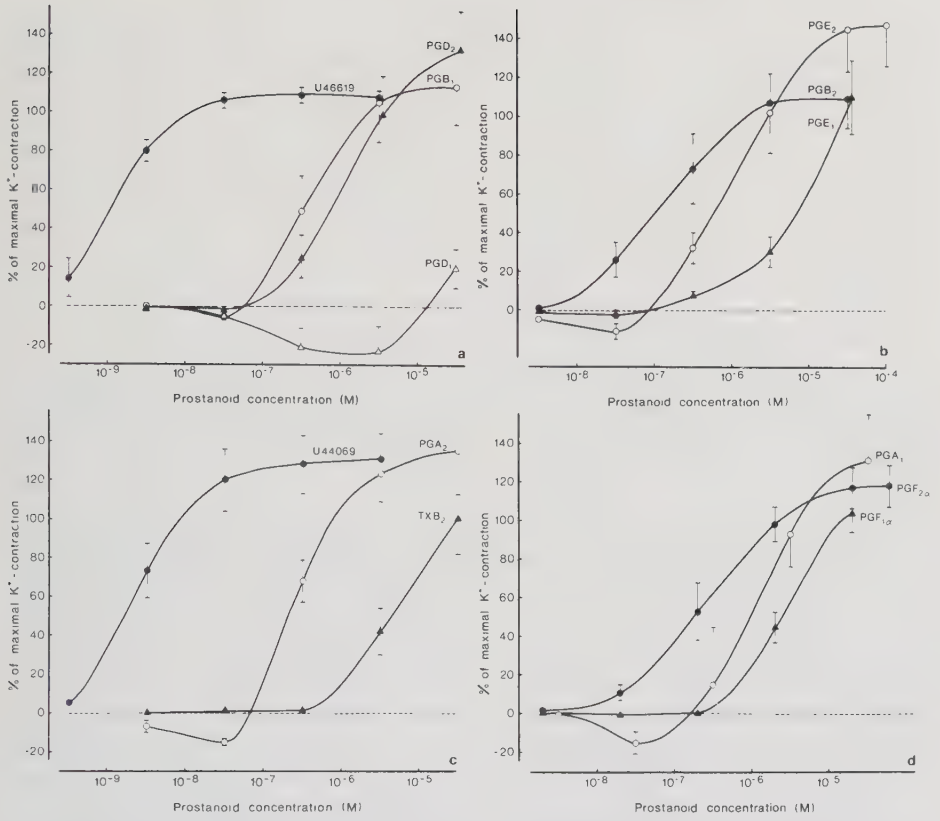

Fig. 1. Responses (mean values ±SE) to various prostanoids of the feline basilar artery at low resting tension expressed as % of the maximal K^+-induced contraction. (*a*) Responses to U46619 (●), PGB_1 (○), PGD_2 (▲) and PGD_1 (△); n=5, 5, 6, and 6 respectively. (*b*) Responses to PGB_2 (●), PGE_2 (○) and PGE_1 (▲); n=6, 8, and 6 respectively. (*c*) Responses to U44069 (●), PGA_2 (○) and TXB_2 (▲); n=5, 5, and 5 respectively. (*d*) Responses to $PGF_{2\alpha}$ (●), PGA_1 (○) and $PGF_{1\alpha}$ (▲); n=7, 5, and 5 respectively.

tration-response curves obtained with either PGE_2, $PGF_{2\alpha}$ or U46619.

In order to study relaxant responses to the A, D and E-prostaglandins, the arteries were contracted by either 124 mM K^+ or $PGF_{2\alpha}$ (2×10^{-5} M). When the contraction had stabilized the relaxation-producing substances were added in a cumulative manner. None of the tested PG's relaxed K^+-contracted vessels. When contractions were induced by $PGF_{2\alpha}$ (2×10^{-5} M) a marked relaxant response was elicited only by PGE_1 (Fig. 2). The EC_{50}-value of PGE_1 was $(2.1\pm1.4)\times10^{-6}$ M. As shown by the E_m-values in Table 2, PGE_1 produced a relaxation of approximately 50%; the effects of the other PG's were 13% or less.

To determine whether the contractile effect of $PGF_{2\alpha}$ was dependent on NA release, further experiments were performed. Incubation of the arteries for 15 min with phentolamine (3×10^{-6} M), failed to affect $PGF_{2\alpha}$-induced contractions. $PGF_{2\alpha}$ was able to induce further contractions in vessels contracted by 124 mM K^+ (n=6) or 10^{-5} M NA (n=5) (Fig. 3). In $PGF_{2\alpha}$-contracted vessels, depolarization by excess K^+ (124 mM) induced additional contraction (n=4), whereas NA (10^{-5} M) (n=6) had little effect (Fig. 3).

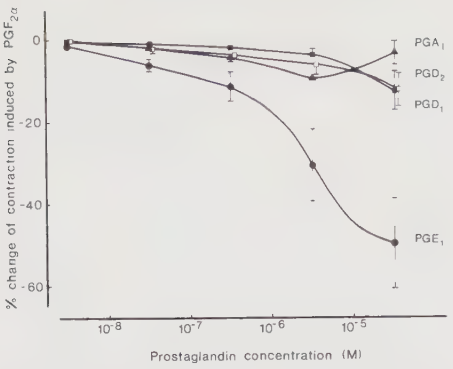

Fig. 2. Relaxant responses (mean values ± SE) to some prostaglandins of vessels contracted by $PGF_{2\alpha}$. The responses are expressed as % reduction of the $PGF_{2\alpha}$-induced contraction. PGA_1(▲), PGD_1(■), PGD_2(□), and PGE_1(●); n=4, 4, 4, and 5 respectively.

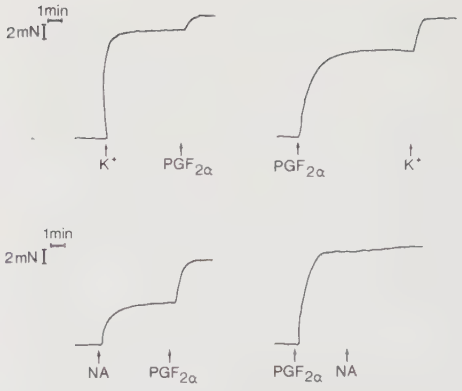

Fig. 3. Tracings illustrating the effects of $PGF_{2\alpha}$ on potassium (K^+)- or noradrenaline (NA)-contracted vessels and the effects of K^+ and NA on $PGF_{2\alpha}$-contracted vessels.

DISCUSSION

The contractile effects obtained with U46619, U44069 and $PGF_{2\alpha}$ in the present study are in good agreement with previously reported results (Dutton et al. 1981, Paul et al. 1982, White & Hagen 1982). The main effects of PGA_1 and PGA_2 were contractile. These PG's have been reported to induce contraction in vitro (Allen et al. 1974 and 1976, Boullin et al. 1976, Yashon et al. 1977), but also to dilate cerebral vessels in vivo (Nakano et al. 1973, Handa et al. 1974). PGE_2, PGD_2 and to some extent PGE_1 also contracted the preparations. The reported effects of these PG's are divergent (Pickard 1981, White & Hagen 1982), which to some extent may be due to species differences (Pickard 1981, White & Hagen 1982, Uski et al. 1983), but also to differing experimental conditions (Pickard 1981, Uski et al. 1981, White & Hagen 1982). In the present study PGB_1 and PGB_2 induced contractions, PGB_2 being more potent than, and PGB_1 equipotent with $PGF_{2\alpha}$. To the best of our knowledge the effect of B-prostaglandins have not previously been studied on isolated brain vessels, but they are potent constrictors of mouse pial arteries in vivo (Rosenblum 1975). PGB_2 also contracted the canine basilar artery in vivo (White 1979).

Bimodal responses, with a relaxation at low concentrations followed by contraction at high, were produced by some of the PG's. Similar responses have been demonstrated with PGI_2 (prostacyclin) on cerebral arteries from dog (Chapleau & White 1979, Chapleau et al. 1980) baboon (Jarman et al. 1979) and man (Paul et al. 1982, Uski et al. 1983), with PGE_2 on the feline middle cerebral artery (Uski et al. 1981), and with various PG's on other vascular preparations in vitro and in vivo (Ogletree, et al. 1978, Borda et al. 1979, Fara et al. 1979, Hatano et al. 1981, Scholtholt et al. 1981).

Thromboxane A_2 (TXA_2) is one of the most potent cerebral vasoconstrictors known (Ellis et al. 1977). However, it is unstable in aqueous solution with a half-life of 30 sec (Hamberg et al. 1975) which complicates detailed studies. There is evidence in support of the view that U46619 acts as a TXA_2-agonist, and it has been proposed that this stable artificial prostanoid may be an acceptable substitute for authentic TXA_2 in pharmacological studies. U46619 has been used as such in the characterization of PG-receptors (Coleman et al. 1980*b*, Kennedy et al. 1982). The order of contractile po-

Table 2. *Relaxant responses of $PGF_{2\alpha}$-contracted vessels to some prostaglandins*

The responses are expressed as % reduction of $PGF_{2\alpha}$-induced contractions

Agent	PGA_1	PGA_2	PGD_1	PGD_2	PGE_1	PGE_2
E_m (%)	10±1	4±0.5	13±4	13±4	50±11	7±2
n	4	4	4	4	5	4

tency obtained in the present study is in agreement with the characteristics of the suggested TX-sensitive type of receptor (TP) (Kennedy et al. 1982). In a previous study, we found that the contractile potency of PGI_2 in the feline BA was similar to that presently found for PGE_2 (Uski et al. 1983). This is also in accordance with the assumption that the receptor mediating contractions in the feline BA is of a TX-sensitive type (Kennedy et al. 1982). However, the concentration-response curves obtained with different prostaglandins displayed different slopes, which may indicate the presence of more than one receptor population.

It has been proposed that SC 19220 has a selective action on one type of PGE-sensitive receptor (EP_1) mediating contraction (Kennedy et al. 1982). We found that SC 19220 did not affect responses to U46619, $PGF_{2\alpha}$ or PGE_2. Providing that SC 19220 has a preference for PGE-receptors this finding suggests either that the possible second contractile receptor is not of a PGE-sensitive type, or that it may be characterized as EP_2 according to the classification of Kennedy et al. (1982). None of the tested PG's were able to relax K^+-contracted preparations. In $PGF_{2\alpha}$-contracted vessels, only PGE_1 had a distinct relaxant action. However, PGE_1 seems to be less potent than PGI_2 under identical conditions (Uski et al. 1983). The effects of the other tested PG's were small (<13%).

Phentolamine failed to affect responses to $PGF_{2\alpha}$. This makes it unlikely that $PGF_{2\alpha}$-effects are mediated through the release of NA from perivascular nerves and subsequent stimulation of α-adrenoceptors. Furthermore, $PGF_{2\alpha}$ was able to induce further contractions in preparations contracted by K^+ or NA. Similar results have been reported in other preparations (Mikkelsen & Andersson 1978), and suggest that $PGF_{2\alpha}$ induces contraction by mechanisms at least partly different from those of these agents.

In conclusion, PG-induced contraction of the feline BA seems to be mediated by a TX-sensitive receptor. The contractile effects of $PGF_{2\alpha}$ do not seem to involve release of NA from perivascular nerves, but appear to be mediated by mechanisms different from those of K^+ and NA.

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Effects of prostanoids on isolated feline cerebral arteries

II. Roles of extra- and intracellular calcium for the prostaglandin $F_{2\alpha}$-induced contraction

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USKI, T. K. & ANDERSSON, K.-E.: Effects of prostanoids on isolated feline cerebral arteries. II. Roles of extra- and intracellular calcium for the prostaglandin $F_{2\alpha}$-induced contraction. Acta Physiol Scand 1984, 120: 197–205. Received 17 May 1983. ISSN 0001-6772. Department of Clinical Pharmacology, University Hospital of Lund, Sweden.

The roles of extra- and intracellular calcium for the contractile effects of $PGF_{2\alpha}$ in the feline basilar artery (BA) were investigated. Comparisons were made with contractions induced by K^+ and noradrenaline (NA). Addition of nifedipine to $PGF_{2\alpha}$- or K^+ (124 mM)-contracted arteries resulted in an incomplete relaxation, whereas NA-contracted vessels were completely relaxed. Incubation of the preparations in a calcium-free medium containing 10^{-5} M EGTA for 5–10 min almost abolished contractions induced by K^+ and NA. In contrast, 63 % of the response to $PGF_{2\alpha}$ remained after pretreatment of the arteries in a calcium-free solution for 40 min; $PGF_{2\alpha}$ produced a biphasic contraction in 17 out of 20 preparations consisting of a rapidly developing initial phase followed by a second increase in tension after 1–6 min. The second phase was absent if the EGTA-concentration was increased to 10^{-4} M, or if the arteries were pre-treated with nifedipine. After incubation of the arteries in a calcium-free medium for 40–120 min and K^+-depolarization, re-addition of calcium elicited contractions at lower concentrations in the presence of $PGF_{2\alpha}$ than in controls. The results suggest that $PGF_{2\alpha}$-induced contractions in the feline BA are considerably less dependent on extracellular calcium than contractions evoked by K^+ or NA. $PGF_{2\alpha}$ appears to be able to release calcium from two cellular stores, and may also promote calcium influx through the cell membrane.

Key words: $PGF_{2\alpha}$, calcium-free medium, nifedipine, excitation-contraction coupling, isolated basilar arteries, cat

Much attention has presently been directed towards the use of calcium channel blockers (or "calcium antagonists") in attempts to alleviate or prevent cerebral ischemic complications due to delayed cerebral vasospasm after subarachnoid hemorrhage (Allen & Bahr 1979, Allen & Banghart 1979, Edvinsson et al. 1979, Shimizu et al. 1980, Brandt et al. 1981 and 1983, Auer et al. 1982, Kazda & Towart 1982, White et al. 1982, Allen et al. 1983). The rationale behind these attempts is to block the calcium influx to the cell and thereby interfere with the final common pathway for cerebral vasoconstriction. Calcium plays a central role in muscle contraction and the crucial step in the activation of the contractile proteins is the increase of the free intracellular calcium concentration. This can be achieved either by influx of calcium through the cell-membrane, by release of cellularly stored calcium, or both (Fleckenstein 1977, Bolton 1979, Godfraind 1981).

There is evidence supporting the view that prostaglandins (PG's) are able to release calcium from intracellular stores (Carsten 1973 and 1974, Malmström & Carafoli 1975, Bolton 1979, McNamara et al. 1980, Loutzenhiser & van Breemen 1981) and that this is an important mechanism for the contractile effects of these agents (Mikkelsen & Andersson 1978, Bolton 1979, McNamara et al. 1980, Wheeler & Weiss 1980, Loutzenhiser & van Breemen 1981). On the other hand, there are studies showing that the contractile effect of PG's on vascular smooth muscle from some regions is dependent mainly on

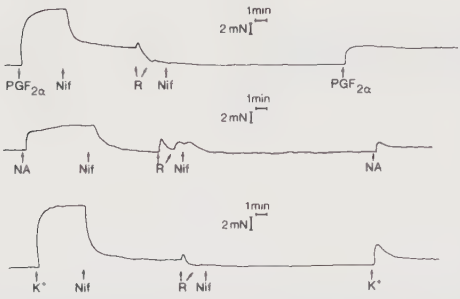

Fig. 1. Tracings illustrating the relaxant effect of nifedipine (NIF) on $PGF_{2\alpha}$, noradrenaline (NA) or potassium (K^+)-contracted vessels. The ability of nifedipine to inhibit contractions induced by these agents is also demonstrated. R indicates rinse of the bath.

the influx of extracellular calcium (Smith et al. 1981, Godfraind & Miller 1982). PG's have been proposed as mediators of cerebral vasospasm (White 1979). Since the effects of PG's on the excitation–contraction coupling (EC-coupling) in cerebral vessels have not been established, it is not known whether blocking calcium influx effectively can prevent PG-induced contractions.

The present study was performed in order to obtain further information on the roles of extra- and intracellular calcium for the contractile effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in feline cerebral arteries. For this purpose, we have studied the effects of extracellular calcium deprivation and calcium channel blockade with nifedipine on responses induced by $PGF_{2\alpha}$. For comparison, corresponding experiments were performed with K^+ and noradrenaline (NA) as contractile agents.

MATERIALS AND METHODS

Preparation and recording of mechanical activity

The methods for preparation and registration of mechanical activity have been described previously (Uski & Andersson 1983). Briefly, basilar arteries were obtained from adult cats exsanguinated under sodium pentobarbital anaesthesia. Ring segments, approximately 2–3 mm long, were suspended between metal prongs in 5 ml organ baths containing Krebs solution. This solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. One of the prongs was connected to a force transducer (Grass Model FT 03C) for registration of isometric tension and the output was recorded on a polygraph (Grass Model 7 B).

Solutions

The following solutions were used: (1) "Normal" Krebs solution with the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11.0. (2) Depolarizing solutions containing 124 mM potassium (K^+) were obtained by exchanging NaCl with equimolar amounts of KCl. (3) Calcium-free solutions were obtained by omitting $CaCl_2$ and adding either of 10^{-5}, 10^{-4} or 10^{-3} M EGTA [ethyleneglycol bis(β-aminoethylether)-N,N,N′N′-tetra-acetic acid].

Drugs

$PGF_{2\alpha}$ was supplied as an aqueous solution (Amoglandin®, Astra, Sweden) and was stored at 4°C. Dilutions were made fresh each day with phosphate buffer at neutral pH, and were kept in the refrigerator until immediately prior to use. Nifedipine (Adalat®, Bayer AG) was supplied dissolved at a concentration of 0.1 mg/ml in ampoules containing 15% ethanol, 15% polyethylene glycol and water as solvents. Due to the risk of light-induced degradation of the substance, it was constantly protected from exposure to direct illumination. In separate experiments it was shown that the solvents, in concentrations attained in the bath, had no effect *per se*. Noradrenaline (L-arterenol hydrochloride) was obtained from Sigma. It was dissolved in 0.9% NaCl with 1 mM ascorbic acid (as an antioxidant) and diluted with the same solvent. The concentrations of the agents are given as the final concentrations of the free acid or base in the organ bath.

Experimental procedure

In order to study the dependence on extracellular calcium, the responses to $PGF_{2\alpha}$, NA, and 124 mM K^+ were studied after varying incubation times in calcium-free solution containing 10^{-5}–10^{-3} M EGTA. Prior to this, reproducible contractions (±10%) induced by the agent studied were required before a preparation was accepted for further experiments. All subsequent contractile responses were expressed as per cent of this contraction. Contractions were elicited repeatedly in calcium-free medium and after re-addition of calcium.

During the incubation in calcium-free medium, the buffer solution was changed every 5–10 min. After incubation in calcium-free solution containing 10^{-5} M EGTA for 40–120 min, the effect of a stepwise increase of the extracellular calcium (0.01–4.44 mM) concentration was studied. The contractions thus obtained were expressed as described above. In experiments where the relaxant response to nifedipine was studied in contracted arteries, relaxation was expressed as per cent reduction of the contraction induced.

Statistical analysis

Student's *t*-test (two-tailed) for unpaired data was used for the statistical analysis. The 0.05 level of probability was accepted as significant. When indicated in figures, levels of probability (p) <0.05, 0.01 and 0.001 are represented by *, ** and *** respectively. Whenever appropriate, the results presented in text, tables or figures are expressed as mean values ± standard error of mean (SE), n=number of experiments.

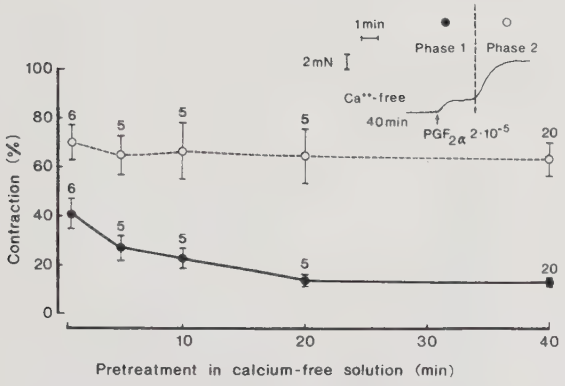

Fig. 2. The effect of varying incubation time in calcium-free solution on responses to $PGF_{2\alpha}$. Contractions (mean ± SE) in calcium-free solution are expressed as % of the contraction obtained in calcium-containing solution. The number of experiments are given above the bars indicating SE. The inset shows a representative tracing demonstrating the contraction induced by $PGF_{2\alpha}$ in calcium-free solution. Closed circles represent the first phase whereas open circles represent the total contractile effect of both phases. For further details: see text.

RESULTS

Effects of nifedipine

Addition of nifedipine (3×10^{-6} M) to $PGF_{2\alpha}$ (2×10^{-5} M)- or K^+ (124 mM)-contracted preparations resulted in an incomplete relaxation (76±2%; n=6 and 81±6%; n=4, respectively, Fig. 1). In contrast, NA (10^{-5} M)-contracted vessels were completely relaxed (109±1.5%; n=4, Fig. 1). Relaxation to levels below resting tension was consistently observed. After nifedipine pre-treatment (3×10^{-6} M) for 15 min, exposure to $PGF_{2\alpha}$ resulted in a sustained, but depressed contraction. NA and K^+ caused non-sustained contractions with reduced amplitudes (Fig. 1.)

Effects of $PGF_{2\alpha}$ in calcium-free medium

The dependence on extracellular calcium of the effects of $PGF_{2\alpha}$ (2×10^{-5} M), K^+ (124 mM) and NA (10^{-5} M) were estimated by examining the responses to these agents after incubation of the preparations in calcium-free solution containing 10^{-5} M EGTA for various times. $PGF_{2\alpha}$ elicited a biphasic response after incubation for 5 min or longer (Figs. 2 and 4): a rapid initial contraction of low amplitude (phase 1) was followed by a second increase in tension (phase 2). Usually, the second phase appeared within 1–2 min after the first contraction had reached its maximum, but the delay was sometimes as long as 5–6 min. After incubation

Table 1. *Effects of varying incubation times in calcium-free solution on contractions induced by $PGF_{2\alpha}$ (2×10^{-5} M), noradrenaline (NA, 10^{-5} M) or K^+ (124 mM)*

The responses are expressed as % of the contraction induced in calcium-containing solution. *p* indicates level of probability between responses induced by $PGF_{2\alpha}$ and K^+ or NA respectively (Student's *t*-test)

	Time in calcium-free medium							
	1 min	n	5 min	n	10 min	n	40 min	n
K^+	29±6	5	10±6	5	5±2	5	3±1	5
p	<0.01		<0.001		<0.001		<0.001	
$PGF_{2\alpha}$	70±7	6	65±8	5	67±12	5	65±7	20
p	<0.001		<0.001		<0.001		<0.001	
NA	9±2	5	8±4	5	0.5±0.2	5	1±0.5	5

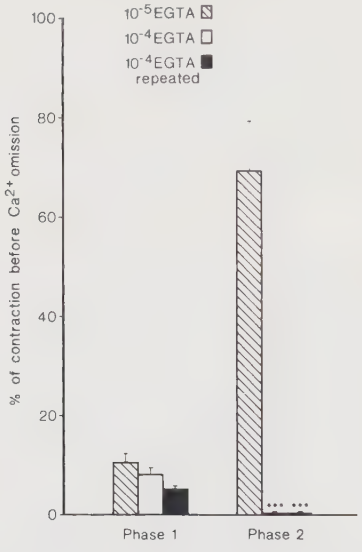

Fig. 3. Graphical presentation of the responses (mean ± SE) to 2×10^{-5} M $PGF_{2\alpha}$ on the feline BA pretreated in calcium-free solution with varying EGTA-concentrations. Filled columns represent the response to $PGF_{2\alpha}$ obtained after several subsequent contractions in high EGTA-solutions after the contraction no longer declined. Contractions are expressed as % of the contraction obtained by $PGF_{2\alpha}$ in calcium containing solution, n=6 in all experiments. Phase 2 was measured as the increase in tension from phase 1.

in calcium-free medium for 40 min, the second phase was absent in 3 out of 20 preparations. Even if these 3 experiments are included 63±7% (n=20) of the response to $PGF_{2\alpha}$ remained after 40 min in calcium-free solution (Fig. 2, Table 1). In contrast, contractions induced by 124 mM K^+ or NA were almost abolished after about 5–10 min in calcium-free medium (Table 1). When contractions could no longer be induced by K^+ or NA, biphasic contractions could still be induced by $PGF_{2\alpha}$.

In arteries responding to $PGF_{2\alpha}$ (2×10^{-5} M) with a biphasic contraction after incubation in calcium-free medium, the response could be repeated at least 2–4 times without intervening calcium exposure. A slight decrease in amplitude was noted, but the response remained biphasic. However, if, after incubation for 40 min in calcium-free medium containing 10^{-5} M EGTA, the EGTA-concentration was increased to 10^{-4} M, the second phase of the $PGF_{2\alpha}$-induced contraction disappeared (Fig. 3). There was also a slight depression of the first phase (non-significant). Repeated contractions led to a further depression of the first phase (Fig. 3), but after 3–4 succeeding contractions no further decrease in the amplitude was observed, not even if the EGTA concentration was increased to 10^{-3} M. Omission of both calcium and magnesium from the bath medium did not further depress the $PGF_{2\alpha}$-induced contraction.

Effects of nifedipine on $PGF_{2\alpha}$-induced responses in calcium-free medium

After more than one hour in calcium-free solution and subsequent depolarization of the arteries by 124 mM K^+, PGF_α (2×10^{-5} M) elicited responses similar to those described above. After K^+-depolarization the amplitude of the first contraction phase increased in half of the preparations (Fig. 4).

Pre-treatment with nifedipine (3×10^{-6} M) for 15 min after incubation in calcium-free medium for at least 40 min, abolished the second phase of the $PGF_{2\alpha}$-induced response (Figs. 4 and 5). The first phase was not significantly depressed. However, if the vessels were also depolarized by 124 mM K^+ (Figs. 4 and 5) the first phase was increased, as compared to the contraction induced by $PGF_{2\alpha}$ alone. The second phase still was significantly depressed by nifedipine.

Effects of calcium re-addition

After incubation in calcium-free medium containing 10^{-5} M EGTA for sufficient time to abolish K^+ (124 mM)- and NA (10^{-5} M)- induced contractions and markedly (contraction less than 10% of control) depress responses to $PGF_{2\alpha}$ (2×10^{-5} M), the effect of a stepwise increase in extra-cellular calcium was examined with one of these agents present. The incubation time in calcium-free medium was never allowed to be shorter than 40 min. In order to depress $PGF_{2\alpha}$-induced contractions sufficiently, incubation in calcium-free solution containing 10^{-4} M EGTA and repeated contractions were needed. The total incubation time in calcium-free solution not infrequently exceeded 2 hours. After this procedure, some residual contractions could still be obtained with $PGF_{2\alpha}$. These contractions by $PGF_{2\alpha}$ were more pronounced in depolarized vessels.

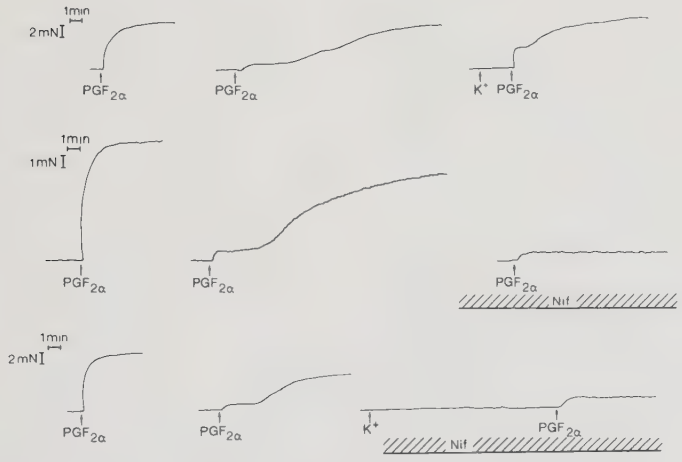

Fig. 4. Tracings demonstrating the effect of 124 mM potassium (K^+), 3×10^{-6} M nifedipine (NIF) or a combination of these agents on the response to $PGF_{2\alpha}$ (2×10^{-5} M) in calcium-free solution. In all tracings the first response is obtained in normal (calcium-containing) solution whereas the succeeding responses are obtained in calcium-free solution containing 10^{-5} M EGTA.

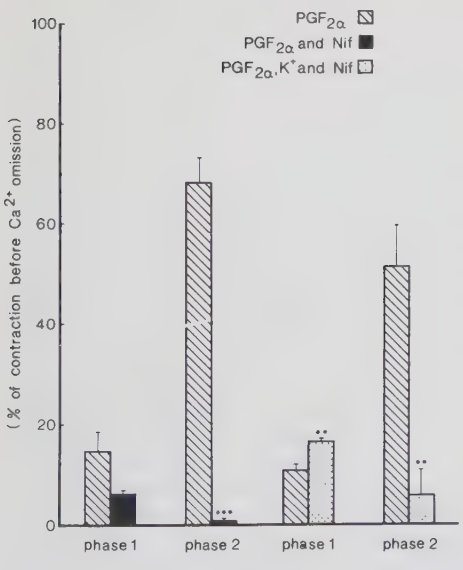

Fig. 5. Graphical presentation of the results obtained in experiments presented in Fig. 4. Reponses (mean ± SE) are expressed as in Fig. 3, n=5, in all experiments.

In the presence of $PGF_{2\alpha}$ addition of calcium (0.01–4.44 mM) induced contractions of the vessels. These could be significantly, but not completely, inhibited if the vessels were also pre-incubated with nifedipine (3×10^{-6} M) for 15 min (Fig. 6). Contractions also occurred when calcium was added in the presence of NA. However, these preparations relaxed in response to calcium concentrations exceeding 1 mM (3 exp.) or 0.1 mM (2 exp.) and, therefore, cumulative concentration-response curves could not be drawn. When calcium was added to K^+-depolarized vessels, contractions occurred (Fig. 7), which were almost abolished by pre-treatment with nifedipine (Fig. 8). In K^+-depolarized preparations calcium elicited contractions at significantly lower concentrations in the presence of $PGF_{2\alpha}$ than in controls (Fig. 7). Calcium-induced contractions in nifedipine-pretreated depolarized vessels also occurred at lower concentrations in the presence of $PGF_{2\alpha}$ (Fig. 8).

DISCUSSION

An increase of the cytoplasmatic calcium concentration is considered necessary for contraction of

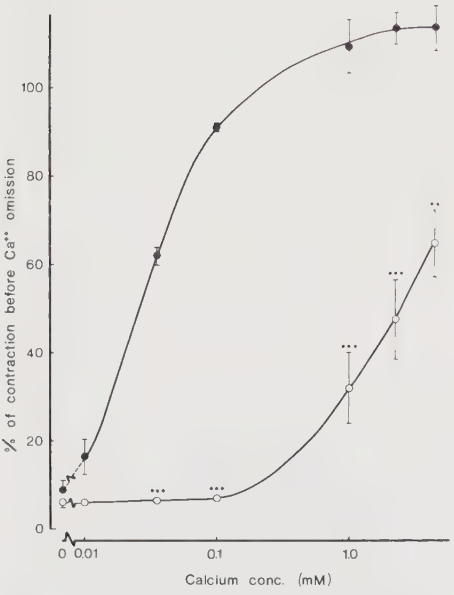

Fig. 6. Effects of increasing extracellular calcium-concentration on the feline BA activated by $PGF_{2\alpha}$ (●), or $PGF_{2\alpha}$ after nifedipine (NIF)-pretreatment (○) in calcium-free solution. Responses (mean ± SE) are expressed as % of the contraction obtained in calcium-containing solution, n=4 and 5 respectively. The contractions obtained in calcium-free medium before calcium-readmission are indicated on the ordinate. When performing significance-tests (Student's *t*-test), correction was made for differences in tension before calcium-admission.

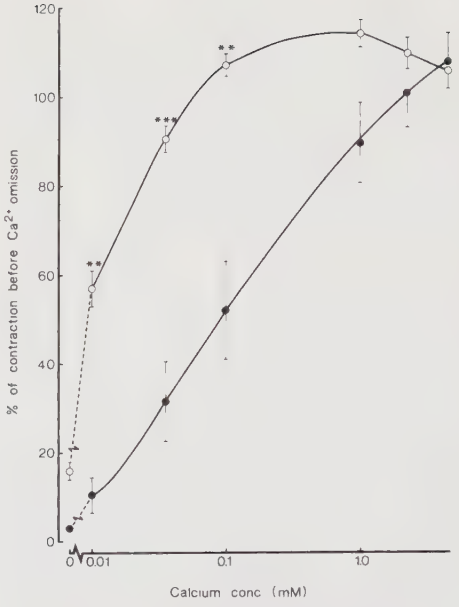

Fig. 7. Effects of increasing extracellular calcium-concentration on the feline BA activated by K^+ (●) or K^+ and $PGF_{2\alpha}$ (○) in calcium-free solution. Responses are expressed as in Fig. 6, n=5 and 5 respectively. The contractions obtained before calcium-readmission are indicated on the ordinate. When performing significance-tests correction was made for differences in tension before calcium-admission.

smooth muscle. The increase can be achieved either by the release of cellularly bound calcium or by the influx of calcium from the extracellular medium (Fleckenstein 1977, Bolton 1979, Godfraind 1981). It has been demonstrated that PG's are capable of releasing calcium from intra-cellular stores (Carsten, 1973–1974, Malmström & Carafoli 1975, Bolton 1979, McNamara et al. 1980, Wheeler & Weiss 1980, Loutzenhiser & van Breemen 1981), but there are also results favouring the view that their contractile effect is mainly dependent on influx of extracellular calcium (Smith et al. 1981, Godfraind & Miller 1982). The present results from experiments using calcium-free medium showed that the responses to $PGF_{2\alpha}$ were much less dependent on extracellular calcium than the responses to K^+ or NA. In fact, a substantial part of the $PGF_{2\alpha}$-induced contraction appeared to be mediated through the release of cellularly bound calcium.

$PGF_{2\alpha}$ usually elicited a biphasic response in calcium-free solution containing 10^{-5} M EGTA. A first contraction of low amplitude was followed by a second increase in tension. This makes it tempting to speculate that the contraction in calcium-free medium is mediated by the release of calcium from different cellular stores. There is evidence in favour of the concept that a small increase in free intracellular calcium may subsequently trigger a release of bound calcium (Fabiato & Fabiato 1979). If the arteries were pre-treated with nifedipine the second phase of the contraction was abolished whereas the first phase was still present. Even if nifedipine is considered to exert its main action by blocking the influx of calcium through specific channels in the cell membrane (Fleckenstein 1977, Bolton 1979,

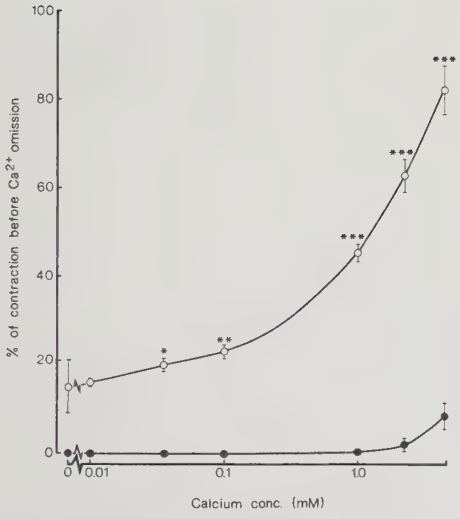

Fig. 8. Effects of increasing extracellular calcium-concentration on the feline BA activated by K^+ (●) or K^+ and $PGF_{2\alpha}$ (○) after nifedipine (NIF)-pretreatment in calcium-free solution. Responses are expressed as in Fig. 6, n=3 and 5 respectively. The contractions obtained before calcium-readmission are indicated on the ordinate. When performing significance-tests correction was made for differences in tension before calcium-admission.

Triggle 1981), there are also observations in favour of an intracellular action (Mikkelsen & Andersson 1978, Bolton 1979, Mikkelsen et al. 1979, Church & Zsotér 1980). Thus, it seems reasonable to assume that $PGF_{2\alpha}$ releases cellularly bound calcium, probably from two different stores, and that the release from one of these stores is blocked by nifedipine.

We observed that the second phase of the $PGF_{2\alpha}$-induced contraction was absent in 3 out of 20 preparations after repeated washings with calcium-free solution for 40 min. The second phase also disappeared if the EGTA concentration in the buffer solution was increased from 10^{-5} M to 10^{-4} M. These observations suggest that the second phase is produced by release of superficially bound calcium. The biphasic contractile response could be reproduced several times in buffer solution containing 10^{-5} M EGTA without intervening calcium re-addition. This may be due to efficient recycling of the released calcium or to a large amount of calcium being stored. A substantial part of the cellularly bound calcium available for the excitation-contraction coupling (EC-coupling) seems to be stored in the cell membrane, in the membrane vesicles or parts of the sarcoplasmatic reticulum closely related to these vesicles (Gabella 1971–1973, Devine et al. 1973, McGuffee & Bagby 1976). Membrane vesicles can not be considered as strictly intracellular since they are closely related to the cell membrane and also share some characteristics with the extracellular calcium pool (Devine et al. 1973, Gabella 1973). They probably communicate with the extracellular space, but their openings seem to be covered by the glycocalyx layer of the cell membrane (Gabella 1973, Langer, Frank & Philipson 1982).

Even if our data do not allow the conclusion that the calcium responsible for the second phase of the $PGF_{2\alpha}$-induced contraction originates from the cell membrane, three of the features of the biphasic response can be explained by assuming this: 1) The second phase was blocked by nifedipine; 2) The second phase was sometimes absent after repeated washings in calcium-free solution containing 10^{-5} M EGTA, 3) The second phase always disappeared if the EGTA concentration in the buffer solution was increased to 10^{-4} M. Studies on cardiac muscle have shown that zero calcium and EGTA disrupts the glycocalyx layer of the cell membrane (Langer et al. 1982), and, thus, greatly facilitates the removal of calcium from the membrane.

It was possible to induce small contractions by adding $PGF_{2\alpha}$ even after several subsequent exposures to this agent in calcium-free solution containing 10^{-4} M EGTA. Exclusion of magnesium did not further attenuate the residual contractions. Thus, it seems less conceivable that the ion mediating these contractions is magnesium. The remaining contractions could not be abolished even if repeated in a buffer solution containing 10^{-3} M EGTA. Similar results have been reported by Casteels et al. (1981) with NA and histamine on various extracranial rabbit arteries. These authors discussed the possibity that their results might indicate the presence of an EC-coupling mechanism totally independent of calcium. Such a calcium-independent mechanism may account also for a small part of the PG-induced contractions in the feline BA.

In addition to the release from cellular calcium stores, contractions induced by different agents may be mediated by the influx of calcium through specific channels in the cell membrane (Fleckenstein 1977, Bolton 1979). It is generally assumed

that there are two populations of calcium channels: membrane potential sensitive and receptor-operated channels (ROC's) (Bolton 1979, Andersson & Högestätt 1983). Depolarization of the cell (e.g., by K^+) opens membrane potential-operated channels, whereas activation by different agonists may open ROC's, but also depolarize the cell and thereby open membrane potential-operated channels (Bolton 1979, Vanhoutte 1982, Andersson & Högestätt 1983).

Preparations pre-treated in a calcium-free medium and then depolarized by K^+ were concentration-dependently contracted by calcium. If $PGF_{2\alpha}$ was added, the concentration-effect curve was displaced to the left. This observation suggests that $PGF_{2\alpha}$ promotes calcium influx via pathways that differ from the channels opened by potassium. The calcium-induced contractions in depolarized vessels were effectively inhibited by nifedipine. This effect of nifedipine was significantly reduced if $PGF_{2\alpha}$ was present in the bath. Since ROC's are blocked less readily by nifedipine than potential sensitive channels (Bolton 1979, Triggle 1981), these results further support the possibility that $PGF_{2\alpha}$ may open receptor-operated channels.

In conclusion, the present results suggest that a substantial part of the $PGF_{2\alpha}$-induced contraction is relatively independent of free extracellular calcium, and that $PGF_{2\alpha}$ causes release of cellularly bound calcium, probably from two different stores. In addition, $PGF_{2\alpha}$ may promote the entry of calcium through the cell membrane.

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IV

Characterization of the prostanoid receptors and of the contractile effects of prostaglandin $F_{2\alpha}$ in human pial arteries

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The contractile and relaxant effects of various prostanoids were studied on isolated human pial arteries. Contractions were elicited with the following order of potency: U46619 ≈ U44069 > PGB_2 > $PGF_{2\alpha}$ > PGE_2 ≈ PGD_2≈ $PGF_{1\alpha}$ ⩾ TXB_2, indicating that prostanoid-induced contractions probably are mediated by a thromboxane-sensitive receptor. Relaxation of $PGF_{2\alpha}$-contracted arteries was induced with the order of potency: PGE_2 > PGE_1 > PGD_2 PGD_1. Vessels contracted by K^+ were relaxed only by PGE_1. Since PGI_2 was previously found to be more potent than all the prostanoids tested in the present study, relaxant responses are probably mediated via a PGI_2-sensitive receptor. The roles of free extracellular and cellularly bound calcium for the contractile effects of $PGF_{2\alpha}$ and K^+ were estimated by incubating the arteries for various times in calcium-free medium containing 10^{-5} M EGTA. Incubation for 5 - 10 min abolished K^+-induced contractions, whereas after 40 min of incubation $PGF_{2\alpha}$ still induced a contraction that reached 70 % of control. The $PGF_{2\alpha}$-induced contraction was biphasic in 8 out of 10 preparations. The second phase could be eliminated by increasing the EGTA-concentration to 10^{-4} M, as well as by nifedipine pretreatment. In calcium-free, high K^+ medium calcium-induced contractions were elicited at lower concentrations in the presence of $PGF_{2\alpha}$. The results suggest that $PGF_{2\alpha}$-induced contractions in human pial arteries are relatively independent of free extracellular calcium. $PGF_{2\alpha}$ may promote trans-membrane influx of calcium, as well as release calcium from seemingly superficially located cellular stores.

Key words: Human pial arteries, prostanoids, prostanoid receptors, calcium-free medium, nifedipine, excitation-contraction coupling.

INTRODUCTION

Prostanoids may be involved as mediators not only in the normal regulation of cerebral blood flow, but also in several pathological conditions such as cerebral vasospasm (White 1979, Pickard 1981, White & Hagen 1982) and migraine (White & Hagen 1982, Parantainen & Vapaatalo 1983). These substances are syntesized both by cerebral vessels (Hagen et al. 1978, Abdel-Halim 1980) and by brain tissue (Coceani & Pace-Asciac 1976, Abdel-Halim 1980). If prostanoids play a major role in the pathogenesis of delayed cerebral vasoconstriction and ischemic dysfunction, encountered in many patients with subarachnoid hemorrhage due to a ruptured intracranial aneurysm, blocking of the specific receptor sites might theoretically counteract the development of this complication. Thromboxane A_2 (TXA_2), derived from a precursor common to the prostaglandins (PG´s), is one of the most potent cerebral vasoconstrictors (Ellis et al. 1977, von Holst et al. 1982), but also e.g. $PGF_{2\alpha}$, PGB_2 and PGE_2 have contractile effects (White 1979, Pickard 1981, Uski & Andersson 1984 a). Before exploring possible therapeutic approaches involving the counteraction of prostanoid-induced vasoconstriction, a characterization of the prostanoid receptor sites in human cerebral arteries seems warranted. In addition, further information on the effects of prostanoids on the excitation-contraction (EC) coupling process in human cerebral arteries is desirable.

At least three different prostanoid receptors mediating smooth muscle contraction have been suggested: TXA_2-sensitive (TP), $PGF_{2\alpha}$-sensitive (FP), and PGE-sensitive (EP) receptors (Kennedy et al. 1982). There is evidence that prostanoid-induced contractions in the feline basilar artery (BA) are mediated by a TP-receptor (Uski & Andersson 1984 a). This is probably also the case with human pial arteries, which show high sensitivity to the

artificial prostanoid U46619 (Dutton et al. 1981, Uski et al. 1981, Paul et al. 1982), a suggested selective TP-receptor agonist (Coleman et al. 1981, Kennedy et al. 1982).

It has previously been shown that PG-induced contractions in the isolated feline BA are relatively independent of free extra-cellular calcium (Uski & Andersson 1984b). The calcium channel blockers nifedipine and nimodipine were shown to be less effective in relaxing isolated human pial arteries contracted by $PGF_{2\alpha}$ than arteries contracted by potassium (Brandt et al. 1981). In contrast, it has been proposed that PG-induced contractions in some other vascular regions are mainly dependent on extracellular calcium (Smith et al. 1981, Godfraind & Miller 1982). No detailed information concerning the mechanisms by which PG´s affect cellularly bound calcium in human pial arteries seems available.

The present study had two main objectives. One was to establish the relative order of contractile and relaxant potencies of some prostanoids on human pial arteries in order to aquire an indication on the nature of the prostanoid receptors. The other aim was to explore some aspects of the mechanisms behind the contractile effects of $PGF_{2\alpha}$. For this purpose we have studied the influence of calcium-exclusion and nifedipine on the action of this prostanoid.

Material and Methods

Preparation

Human pial arteries were removed together with small parts of adjacent brain tissue before lobe resections were performed in 15 patients with a deeper located glioma. All patients received premedication with diazepam and were operated under general anaesthesia (fentanyl, thiopental and pancuronium). The specimens were promptly immersed in cooled (about + 4° C) Krebs solution (for composition, see below). After arrival to the laboratory (within 10 - 15 min), the arteries were carefully dissected free from surrounding tissue under the microscope and were, thereafter, either used immediately or stored at +4° C for up to 24 h.

Recording of mechanical activity

Ring segments, approximately 300 - 400 μm wide and 2 - 3 mm long, were suspended between two L-shaped metal prongs in temperature controlled organ baths each containing 5 ml of Krebs solution. The methodology has been described in detail previously. Cerebral arteries of a size down to about 100 μm can be mounted without causing any injury to the endothelial lining of the vessels (see Högestätt et al 1983). The bath solution was continuously gassed with 5 % CO_2 in O_2, resulting in a pH of approximately 7.4 and was maintained at 37° C. One of the two metal prongs was connected to a force transducer (Grass Model FT03 C) for registration of isometric tension, and the other prong was fixed to a displacement device allowing precise adjustments of vessel tension. The output from the transducer was amplified by, and recorded on, a polygraph (Grass Model 7B). Each vessel was subjected to a slowly increasing tension reaching a final value of approximately 4 mN during which time the buffer solution was exchanged every 15 min.

Solutions

The following solutions were used:

1) "Normal Krebs" solution containing (mM); NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11.0.

2) Depolarizing solutions containing 124 mM potassium (K^+). These were obtained by exchanging NaCl with equimolar amounts of KCl.

3) Calcium-free solutions. These were obtained by omitting $CaCl_2$ and adding either of 10^{-5} M or 10^{-4} M EGTA (ethyleneglycol bis (β-aminoethylether)-N,N,N´,N´,-tetra-acetic acid).

Drugs

Stock solutions (10 mg/ml) of the following prostanoids were made up in absolute ethanol: PGB_2, PGD_1, PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$, thromboxane B_2 (TXB_2), the prostaglandin endoperoxide analogue U 44069 ((15 S)-hydro-9α, 11 α-(epoxymethano) prosta-5 Z, 13 E-dienoic acid) and the TXA_2 agonist U46619 ((15 S)-hydroxy-11 α, 9 α-(epoxymethano) prosta-5 Z, 13 E-dienoic acid) (Upjohn, USA) (Coleman et al 1981, Kennedy et al 1982). All stock solutions were stored at -20° C. $PGF_{2\alpha}$ was supplied as an aqueous solution (Amoglandin^R, Astra, Sweden) and was stored at +4 °C. All dilutions were made fresh each day with phosphate buffer at neutral pH. Nifedipine was obtained from Bayer AG dissolved in 15 % ethanol, 15 % polyethylene glycol and water at a concentration of 0.1 mg/ml (Adalat^R). To prevent decomposition of nifedipine, the solutions were protected from light exposure except during experiments.

Experimental procedure

All contractile responses were expressed as per cent of contractions obtained with a depolarizing solution containing 124 mM K^+ (8.8 ± 0.7 mN; n=42). On the basis of the results obtained by cumulative adding of the

prostanoids to the bath concentration-response curves were constructed. Relaxant responses to prostaglandins were studied in K^+ and $PGF_{2\alpha}$ contracted arteries, and relaxation was expressed as per cent reduction of the starting tension. Both contractile and relaxant responses were characterized with regard to drug concentrations resulting in half-maximum effect (EC_{50}). The maximum responses (E_m) that could be obtained were also determined.

In order to study the dependence on free extracellular calcium, the effects of $PGF_{2\alpha}$ and 124 mM K^+ were investigated after varying incubation times in calcium-free solution containing 10^{-5} or 10^{-4} M EGTA. Preceding this procedure, reproducible contractions (± 10 %) were obtained with the agent studied, and subsequent contractile effects were expressed as per cent of this contraction. During the incubation in calcium-free medium, the buffer solution was changed at least every 5 - 10 min. After incubation in calcium-free solution for >40 min, the effect of increasing the extracellular calcium concentration (0.01 - 4.44 mM) in a stepwise manner was studied. The contractions thus obtained (expressed as described above) were used for the construction of concentration-response curves for calcium.

Statistical analysis

Student's two-tailed *t*-test was used for statistical comparison between groups of data.The 0.05 level of probability (p) was accepted as significant. When indicated in figures, p-levels of 0.05, 0.01 and 0.001 are represented by *, **, and *** respectively. When appropriate, results presented in text, tables or illustrations are expressed as mean values ± standard error of the mean (SE), n= number of experiments.

RESULTS

Effects of prostanoids

Except for PGD_1 and PGE_1 all prostanoids induced contractions. The following order of potency was obtained: U46619 $\approx$ U 44069 > PGB_2 > $PGF_{2\alpha}$ > $PGE_2 \approx PGD_2 \approx PGF_{1\alpha} \gg TXB_2$. The EC_{50} and E_m-values are listed in table 1. These values could, however, not be adequately determined for PGD_2, PGE_2, $PGF_{1\alpha}$ and TXB_2 since the curves did not reach a plateau within the concentration range studied.

The responses to D- and E-prostaglandins were studied in arteries contracted by either K^+ (124 mM) or $PGF_{2\alpha}$ (2×10^{-5} M). In K^+-contracted arteries PGE_1 had a relaxant effect with an EC_{50}-value of $(1.6 \pm 1.7) \times 10^{-7}$ M and an E_m-value of 17 ± 3 % (n=5), PGD_2 and PGE_2 induced further contractions, whereas PGD_1 was found to be inactive (Fig 2). In $PGF_{2\alpha}$-contracted vessels all these prostanoids had relaxant effects (Fig 3). The order of relaxant potency was : PGE_2 > PGE_1 > $PGD_2 \approx PGD_1$. The EC_{50}- and E_m-values for PGE_1 were $(1.0 \pm 1.2) \times 10^{-6}$ M and 83 ± 2 % (n=5) and for PGE_2 $(5.2 \pm 1.5) \times 10^{-7}$ M and 25 ± 7 % (n=4). The EC_{50} and E_m-values for the D-prostaglandins could not be adequately determined since the curves did not reach a plateau within the concentration range studied.

Influence of calcium-free medium on $PGF_{2\alpha}$- and K^+-elicited responses.

After incubation of the vessel segments for varying periods (1 - 40 min) in calcium-free solution containing 10^{-5} M EGTA, the effects of $PGF_{2\alpha}$ (2×10^{-5} M) and K^+ (124 mM) were studied. $PGF_{2\alpha}$ usually elicited a biphasic contraction, i. e. an initial rapidly developing contraction of low amplitude followed by a second, gradual and marked increase in tension (Figs 4, 6 and 7). Following periods up to 20 min in calcium-free medium, the responses were often less distinctly bi-

phasic (Fig 6). In addition, the second phase was absent in 1 out of 6 preparations after 10 min and in 2 out of 10 preparations after incubation in calcium-free medium for 40 min. After 40 min incubation time in calcium-free medium $PGF_{2\alpha}$ still induced a contraction that reached 70 ± 11 % (n=10) of the response before exclusion of calcium (Fig 5). In contrast, K^+, induced a phasic contraction which rapidly declined already after 1 min incubation in calcium-free medium (Figs 4 and 5) and was almost abolished after 5 - 10 min (Fig 5). In many of these preparations $PGF_{2\alpha}$ induced strong biphasic contractions despite the lack of response to K^+ (Fig 6).

Influence of nifedipine and different EGTA-concentrations on $PGF_{2\alpha}$-induced responses in calcium-free medium

In arteries where $PGF_{2\alpha}$ induced a biphasic or strong monophasic contraction, the response could be repeated at least twice without intervening calcium exposure (Fig 6). A tenfold increase of the EGTA concentration (to 10^{-4} M) significantly depressed the first phase and abolished the second (Figs 7 and 8).

Nifedipine pretreatment (3×10^{-6} M for 15 min) in calcium-free medium (at least 40 min), significantly attenuated the first phase and abolished the second phase of the $PGF_{2\alpha}$-induced response (Figs 6 and 8).

Re-addition of calcium

The effect of calcium-re-addition was studied in arteries incubated in calcium-free medium with 10^{-5} M EGTA for sufficient time to abolish K^+-contractions or markedly depress contractions induced by $PGF_{2\alpha}$. The time in calcium-free medium was never less than 40 min.Incubation in calcium-free solution containing 10^{-4} M EGTA was needed to depress $PGF_{2\alpha}$-induced contractions to less than 15 % of control. In K^+-depolarized vessels,

the contractions were restored by a stepwise re-introduction of calcium to the organ bath. If K^+-depolarized preparations were also exposed to $PGF_{2\alpha}$, re-addition of calcium induced contractions at significantly lower calcium concentrations (Fig 9).

DISCUSSION

There are several previous reports on the contractile effects of $PGF_{2\alpha}$, U46619, U 44069 and PGE_2 in different species and vascular regions (Malik & McGiff 1976, Jones et al.1982, Kennedy et al.1982) including some observations on human cerebral arteries (Dutton et al. 1981, Uski et al. 1981, Paul et al. 1982, White & Hagen 1982). The present study confirms the contractile effects of these agents in human pial arteries. To the best of our knowledge there is no information available concerning the effect of PGB_2 on human cerebral vessels. In this tissue PGB_2 was found to be even more potent than $PGF_{2\alpha}$ as a contractile agent, which agrees with previous findings in the feline basilar artery (BA) (Uski & Andersson 1984a). The order of contractile potency of the prostanoids tested was similar to that previously found in the feline BA (Uski & Andersson 1984a). This supports the concept that the contractile prostanoid receptor in human, as well as in feline pial arteries is of a TXA_2-sensitive type (TP) according to the classification proposed by Kennedy et al. (1982).

The D and E prostaglandins were all capable of relaxing $PGF_{2\alpha}$-contracted arteries, PGE_2 being the most potent. However, the degree of relaxation induced by PGE_1 was much more pronounced than that produced by the other substances. Moreover, only PGE_1 had a relaxant effect in K^+-contracted arteries. We have previously found PGI_2 (prostacyclin) to be almost ten times more potent as a relaxant agent in both K^+ and $PGF_{2\alpha}$-contracted human pial arteries than any of the examined PG´s (Uski et al. 1983). This suggests that in human pial arteries, the relaxation-mediating prostanoid receptor is of a PGI_2-sensitive type, and that it is similar to the receptor described in other vascular preparations (Lumley et al. 1982, Town et al. 1982). Smooth muscle relaxation may, in some vascular regions, also be

mediated by PGE-sensitive receptors (Lumley et al. 1982). Since PGI_2- and PGE-sensitive receptors can coexist in the same tissue (Lumley et al. 1982), the possibility that PG´s of the E-type mediate their relaxant effects via receptors differing from the PGI-sensitive receptors cannot be excluded.

An increase of the free intracellular calcium-concentration is necessary for smooth muscle contraction. This can be achieved either by influx of calcium through specific channels in the cell membrane, by release of cellularly sequestered calcium, or by a combination of these events (Bolton 1979, Godfraind 1981, Andersson & Högestätt 1984). There are indications that release of calcium from intra-cellular stores may mediate the contractile effects of PG´s (Mikkelsen & Andersson 1978, Bolton 1979, McNamara et al. 1980, Wheeler & Weiss 1980, Loutzenhiser & van Breemen 1981). In the feline BA, PG-induced contractions seem to be relatively independent of the free extracellular calcium concentration (Uski & Andersson 1984 b). Similar results have been reported in the canine BA with carbocyclic TXA_2 (Toda 1982), but $PGF_{2\alpha}$-induced contractions seem to be less resistant to extracellular calcium depletion in this preparation (Yamamoto et al. 1983). There are reports that PG-induced contractions in some smooth muscle preparations other than cerebral arteries are highly dependent on calcium in the extracellular medium (Smith et al. 1981, Godfraind & Miller 1982).

The present study demonstrates a relative independency of free extracellular calcium for $PGF_{2\alpha}$-induced contractions in human pial arteries. These results are supported by the finding of Brandt et al. (1981) that human pial vessels were less sensitive to certain calcium entry blockers when contracted by $PGF_{2\alpha}$ than

by K^+. As in the feline BA (Uski & Andersson 1984b), it seems as if $PGF_{2\alpha}$-induced contractions in human pial arteries to a considerable extent are mediated by the release of cellularly bound calcium.

After 40 min pre-incubation in calcium-free medium containing 10^{-5} M EGTA ,the response to $PGF_{2\alpha}$ was biphasic (8 out of 10 preparations). After an increase of the EGTA-concentration to 10^{-4} M, the second phase of the $PGF_{2\alpha}$-induced contraction was always absent, suggesting that this contraction is mediated by a release of superficially bound calcium. Similar results have been reported with $PGF_{2\alpha}$ in the feline BA (Uski & Andersson 1984b) and with noradrenaline in the rat aorta (Heaslip & Rahwan 1982). However, in the latter study the second phase of the contraction was present also after increasing the EGTA-concentration to 10^{-4} M. In the present study the biphasic contraction could be repeated in calcium-free medium with 10^{-5} M EGTA suggesting either that these superficial stores contain relatively large amounts of calcium, or that the released calcium is efficiently recycled.

Several studies indicate that a major part of the cellularly bound calcium is stored in the cell membrane, membrane vesicles or superficially located sarcoplasmatic reticulum (Gabella 1971-1973, Devine et al. 1973, McGuffee & Bagby 1976). The membrane vesicles probably communicate with the extracellular space but seem to be covered by the glycocalyx layer which also coats the lipid bilayer of the sarcolemma (Devine et al. 1973, Gabella 1973, Langer et al. 1982). The glycocalyx coating can be disrupted by calcium exclusion and EGTA (Langer et al. 1982). Since this would greatly facilitate the removal of calcium from the cell membrane, our results with increasing the EGTA-concentration points at the possibility that the calcium

which is released by $PGF_{2\alpha}$ is stored in the membrane. From a recent study on mesenteric arteries it can be deduced that a total incubation time of at least 20 min in calcium-free solution containing 2×10^{-3} M EGTA is needed to remove calcium bound to superficial intracellular stores (Saida & van Breemen 1983). We could abolish the second phase of the PG-induced contraction after only minutes in calcium-free solution containing 10^{-4} M EGTA. If intracellular calcium stores in cerebral arteries are similarly resistant to EGTA, this suggests that calcium released from stores on the outside of the membrane mediates this phase. The first phase was more resistant to EGTA and may thus represent a release from intracellular stores.

There is evidence in favour of the concept that nifedipine exerts its main action by blocking the exterior orifices or "outer mouths" of the calcium channels (Glossman et al. 1982). We found that the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium could be abolished if the vessels were pretreated with nifedipine, an observation identical to previous findings in the feline BA (Uski & Andersson 1984b). This further supports our assumption that a major part of the $PGF_{2\alpha}$-induced contraction in calcium-free medium is mediated via the release of calcium bound to structures on the exterior aspect of the cell membrane, i. e. the glycocalyx layer, the membrane vesicles or binding sites in the lipid bilayer of the membrane. An alternative explanation could be that nifedipine inhibits PG-induced calcium release from intracellular stores (Mikkelsen & Andersson 1978, Church & Zsotér 1980). This interpretation is, however, not supported by our findings with different EGTA-concentrations.

After pretreatment in calcium-free solution and subsequent depolarization by K^+, re-addition of calcium

resulted in concentration-dependent contractions. If $PGF_{2\alpha}$ was added to the depolarized vessels, there was a left shift of the concentration-response curve for calcium. This indicates that $PGF_{2\alpha}$ may promote the entry of calcium through the cell membrane via pathways differing from the calcium channels opened by K^+. In the feline BA, addition of $PGF_{2\alpha}$ enhanced contractions induced by calcium re-admission in depolarized arteries. Furthermore, nifedipine practically abolished the contractile effect of calcium in K^+-depolarized vessels, an effect that was much less pronounced if $PGF_{2\alpha}$ was also present in the bath (Uski & Andersson 1984 b). In addition to supporting the view that different pathways are involved in the transmembrane influx of calcium induced by K^+ and $PGF_{2\alpha}$, this finding also suggests that $PGF_{2\alpha}$ may open receptor operated channels (Bolton 1979).

In conclusion, PG-induced contractions in human pial arteries appear to be mediated via a TX-sensitive (TP) receptor, whereas relaxant effects seem to be mediated by a receptor sensitive to PGI_2. A substantial part of the $PGF_{2\alpha}$-induced contraction is relatively independent of the free extracellular calcium concentration. The present results indicate that $PGF_{2\alpha}$, besides promoting calcium influx through the cell membrane, probably liberates calcium from superficial cellular stores.

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Table 1

Contractile responses to some prostanoids characterized by their EC_{50}- and E_m-values. The contrations are expressed as % of the contraction induced by 124 mM K^+.

Agent	EC_{50}	E_m (%)	n
PGB_2	$(7.0 \pm 1.2) \times 10^{-7}$	89 ± 6	6
$PGF_{2\alpha}$	$(2.2 \pm 1.3) \times 10^{-6}$	97 ± 11	5
U44069	$(7.9 \pm 1.1) \times 10^{-9}$	123 ± 7	5
U46619	$(7.1 \pm 1.5) \times 10^{-9}$	130 ± 8	5

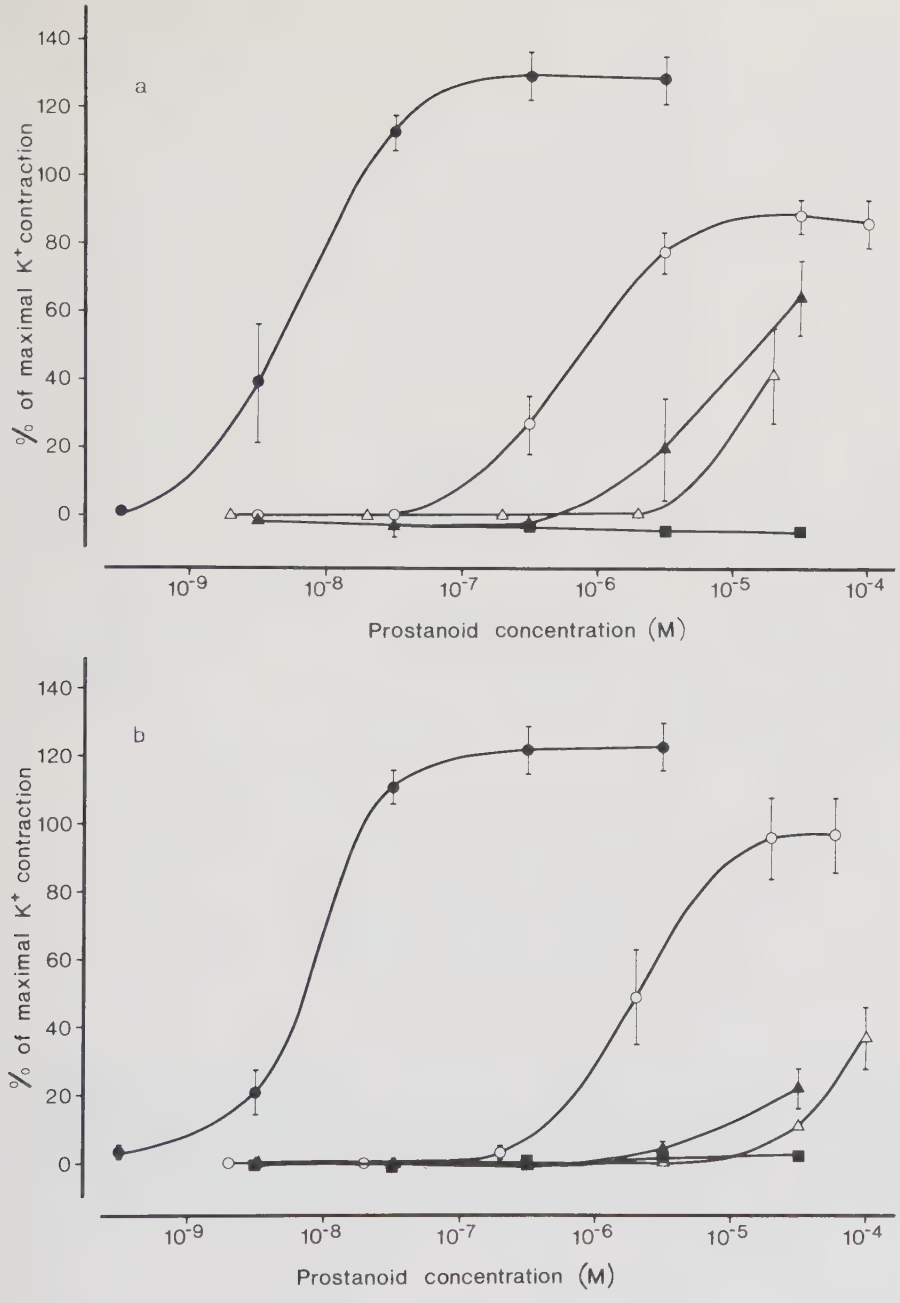

Fig 1. Responses (mean values ± SE) to various prostanoids of human pial arteries expressed as % of the maximal K^+-induced contraction,
a) Responses to U 46619 (●), PGB_2 (o), PGE_2 (▲), $PGF_{1\alpha}$ (△) and PGE_1 (■); n=5,6,5,4 and 3, respectively.
b) Responses to U44069 (●), $PGF_{2\alpha}$ (o), PGD_2 (▲), TXB_2 (△) and PGD_1, (■); n=5,5,5,3 and 3, respectively.

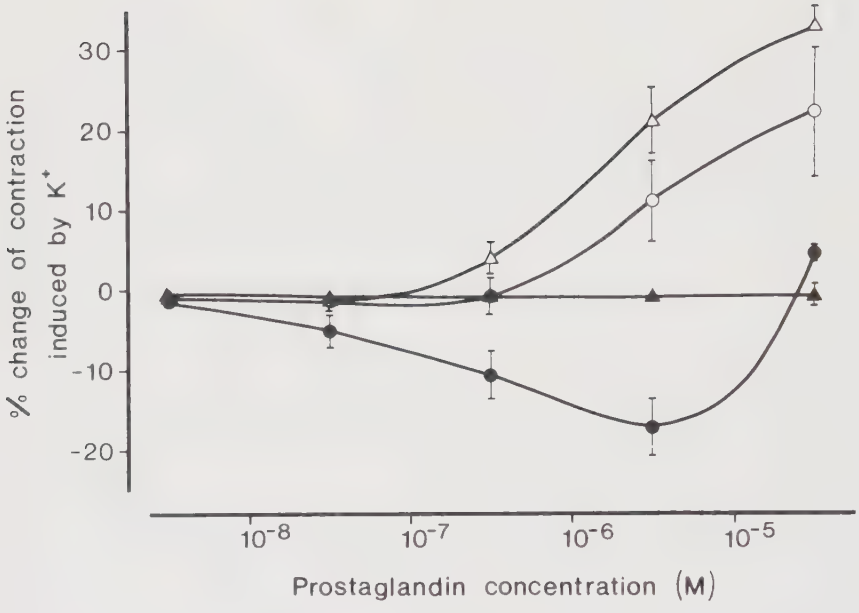

Fig 2. Responses (mean values ± SE) to some prostaglandins of human pial arteries contracted by 124 mM K^+. The responses are expressed as % change of the K^+-induced contraction. PGE_1 (●), PGE_2 (o), PGD_1 (▲) and PGD_2 (△); n=5,4,3 and 4, respectively.

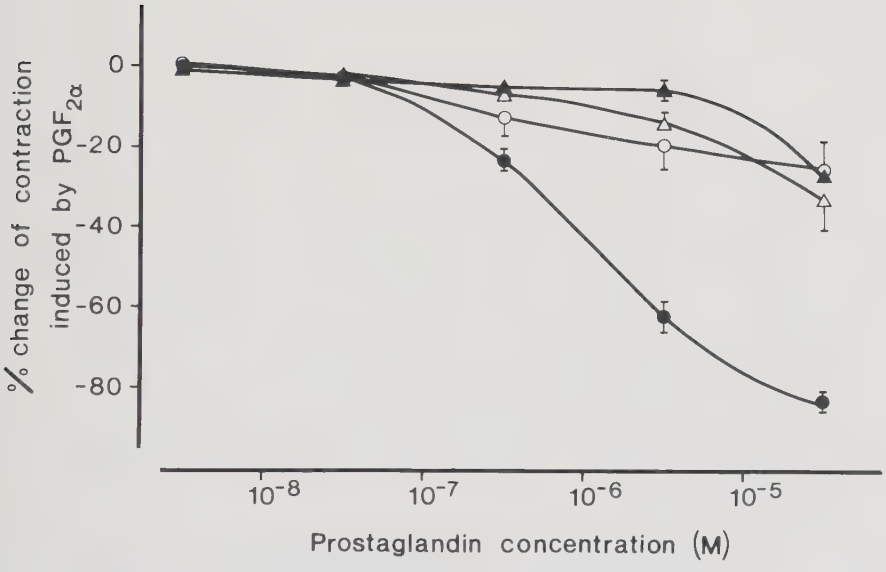

Fig 3. Responses (mean values ± SE) to some prostaglandins of human pial arteries contracted by $PGF_{2\alpha}$. The responses are expressed as % reduction of the $PGF_{2\alpha}$-induced contraction. PGE_1 (●), PGE_2 (o), PGD_1 (▲) and PGD_2 (△); n=5,4,4 and 5 respectively.

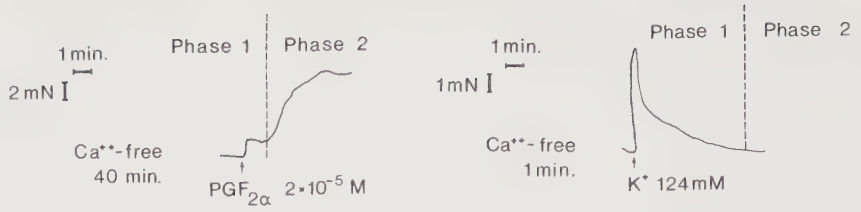

Fig 4. Representative tracing of a contraction induced by $PGF_{2\alpha}$ after 40 min incubation of the vessel in calcium-free solution (left) as compared to a contraction induced by K^+ after 1 min in calcium-free medium (right).

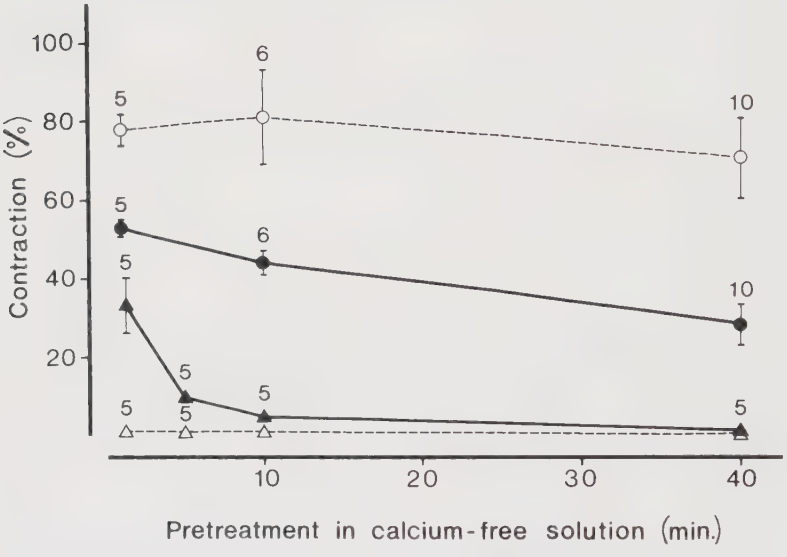

Fig 5. The effect of varying incubation time in calcium-free solution on responses to $PGF_{2\alpha}$ (circles) and K^+ (triangles). Contractions (mean ± SE) in calcium-free solution are expressed as % of the contraction obtained in calcium-containing solution. The number of experiments are given above the bars indicating SE. Closed symbols represent the first phase whereas open symbols represent the total contractile effect of both phases. At each time-point responses to $PGF_{2\alpha}$ differed statistically from responses to K^+ ($p < 0.001$).

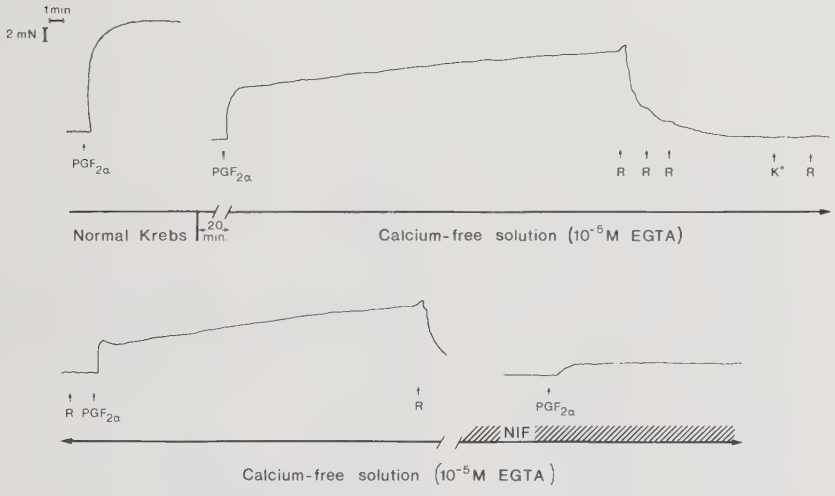

Fig 6. Tracings demonstrating the effect of 3×10^{-6} M nifedipine (NIF) on the response to $PGF_{2\alpha}$ in calcium-free solution. The ability of the vessel to respond to $PGF_{2\alpha}$ by a biphasic contraction, even if the response was repeated without calcium re-admission, as well as the inability of K^+ to induce contractions in calcium-free medium are also demonstrated. Note that the response to $PGF_{2\alpha}$ after 20 min in calcium-free solution was not distinctly biphasic in this preparation.

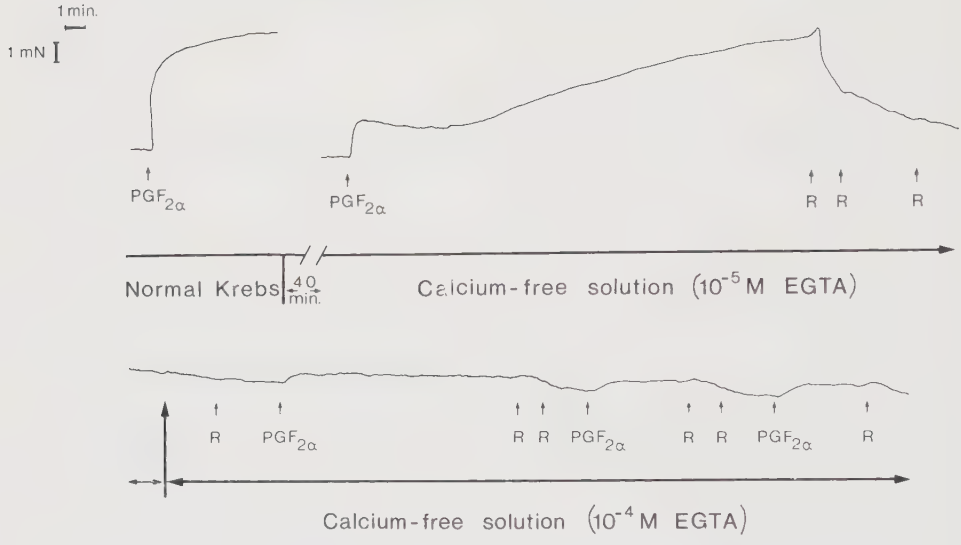

Fig 7. Tracings demonstrating the effect of increasing concentration of EGTA in the calcium-free solution on contractions induced by 2 x 10^{-5} M $PGF_{2\alpha}$. R indicates rinsing of the bath.

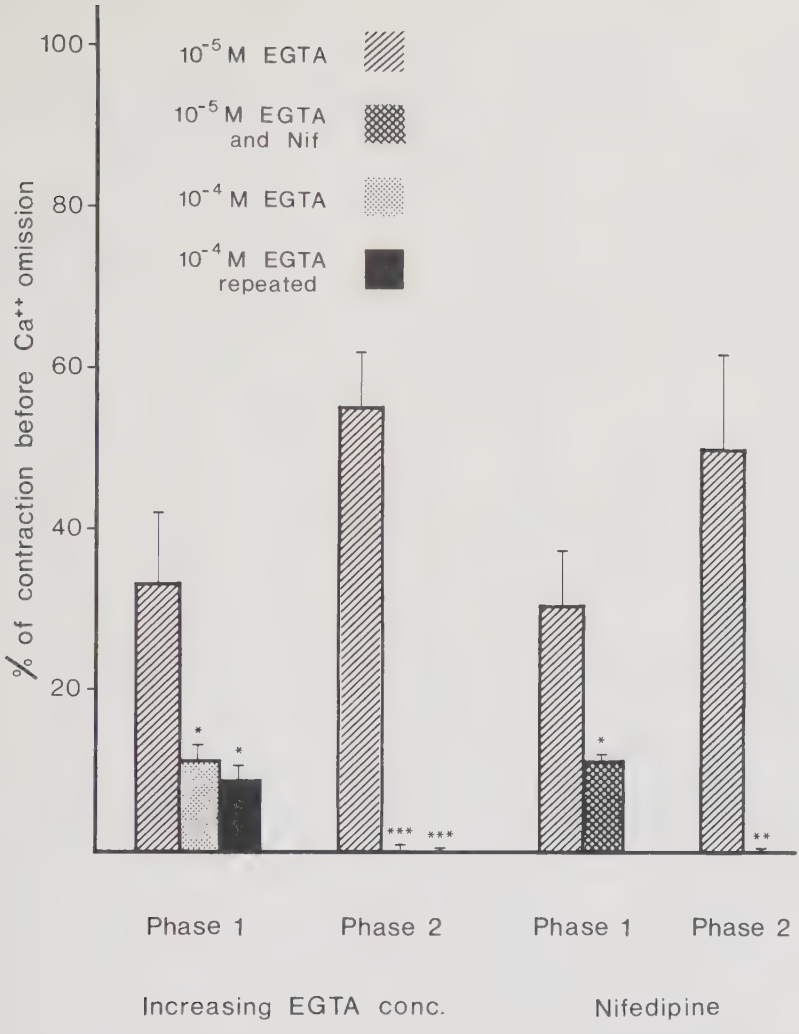

Fig 8. Presentation of the results obtained in the experiments illustrated in Figs 6 and 7. Contractions (mean ± SE) are expressed as % of the contraction obtained by $PGF_{2\alpha}$ in calcium-containing solution(n=5 in EGTA-experiments and n=4 in nifedipine-experiments). Phase 2 was measured as the increase in tension from phase 1.

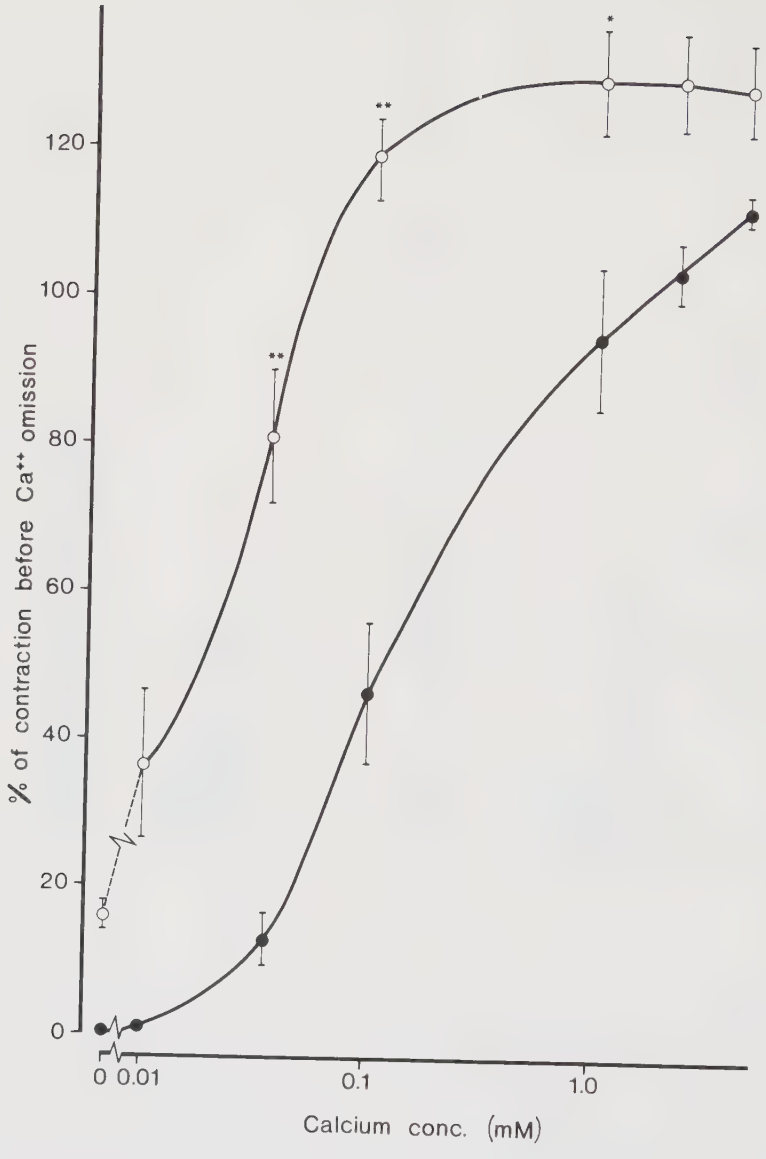

Fig 9. Effects of increasing extracellular calcium-concentration on human pial arteries exposed to K^+ (●) or K^+ and $PGF_{2\alpha}$ (o) in calcium-free solution. Responses (mean ± SE) are expressed as % of the contraction obtained in calcium-containing solution, (n=5). The contractions (if any) obtained in calcium-free medium before re-addition of calcium are indicated on the ordinate. When performing significance-tests, correction was made for differences in tension before calcium-readdition.

V

CELLULAR CALCIUM AND THE CONTRACTION INDUCED BY PROSTAGLANDIN $F_{2\alpha}$ IN FELINE CEREBRAL ARTERIES

by

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Running title: $PGF_{2\alpha}$ and cellular calcium in cerebral arteries.

SUMMARY

1. The influences of different calcium-entry blockers, sialidase and caffeine on the biphasic contraction induced by prostaglandin (PG) $F_{2\alpha}$ in the feline basilar artery (BA) were studied in calcium-free medium.

2. After incubation in calcium-free solution, $PGF_{2\alpha}$ induced a contraction of the BA amounting to 87 % of the contraction in calcium-containing solution. The response was biphasic in 41 out of 42 vessel segments.

3. TRIS-buffered solutions markedly attenuated $PGF_{2\alpha}$-induced contractions as compared to contractions in Krebs solution. $PGF_{2\alpha}$ failed to induce a biphasic contraction (8 out of 9 preparations) in calcium-free HEPES-buffered solution.

4. Calcium entry blockade with 1 mM manganese or 10^{-5} M diltiazem abolished the major second phase of the $PGF_{2\alpha}$-induced contraction.

5. The second contraction phase was also eliminated in four out of five preparations pretreated with sialidase (1 unit/ml for 30 min), but was unaffected by a brief exposure to 20 mM caffeine in calcium-free medium.

6. The present findings strongly support previous suggestions that a major part of the $PGF_{2\alpha}$-induced contraction in calcium-free medium is mediated via the release of calcium bound to the exterior aspect of the cell membrane.

Introduction

There are conflicting results concerning the role of extracellular calcium in prostaglandin (PG) -induced contractions of extracranial vascular smooth muscle (McNamara, Roulet, Gruetter, Hyman & Kadowitz, 1980; Wheeler & Weiss, 1980; Loutzenhiser & vanBreemen, 1981; Smith, Lefer & Nicolaou, 1981; Godfraind & Miller,1982). Contractions induced by carbocyclic thromboxane A_2 in dog basilar artery (BA) (Toda, 1982) were relatively independent of the extracellular calcium concentration, and $PGF_{2\alpha}$-induced contractions in both feline BA (Uski & Andersson, 1984) and human pial arteries (Uski, Andersson, Brandt & Ljunggren, 1984) were considerably more resistant to treatment in calcium-free medium than contractions induced by potassium or noradrenaline. It has been demonstrated that cerebral arteries contracted by $PGF_{2\alpha}$ can be efficiently relaxed by calcium entry blockers (Brandt, Andersson, Edvinsson & Ljunggren 1981; Andersson, Edvinsson, MacKenzie, Skärby & Young 1983; Uski & Andersson, 1984). However, in human cerebral arteries, nifedipine was less effective in counteracting PG-induced contractions than contractions induced by potassium (Brandt et. al., 1981). As the free, intracellular calcium mediating smooth muscle contraction may originate from the extracellular medium or from cellular calcium stores (Bolton, 1979; Godfraind, 1981), these data suggest that $PGF_{2\alpha}$ produces contraction in cerebral arteries mainly by releasing cellular calcium.

$PGF_{2\alpha}$ induced a biphasic contraction in both feline (Uski & Andersson, 1984) and human (Uski et.al., 1984) cerebral arteries pretreated in calcium-free medium. The major second phase could be abolished by nifedipine-pretreatment or by an increase of the EGTA-concentration in the extracellular medium. This was interpreted as indicating that the major part of the PG-induced contraction was mediated by a release of calcium from superficial cellular stores (Uski & Andersson, 1984; Uski et.al., 1984).

The present investigation was performed in order to further study the two phases of the $PGF_{2\alpha}$-induced contraction in calcium-free medium. For this purpose the organic calcium entry blocker diltiazem which probably has another site of action in the membrane than nifedipine (Glossman, Ferry, Lübbecke, Mewes &

Hofmann, 1982 and 1983) and the inorganic calcium entry blocker manganese (Mn^{2+}) were used. The influence of sialidase, which is considered to act on the glycocalyx - an important calcium-binding structure - and the effect of exhaustion of intracellular calcium stores with caffeine were also investigated.

Materials and Methods

Animals

Adult cats were exsanguinated under sodium pentobarbital anaesthesia (30 mg/kg i.p.). The skull was opened and the brain removed. Under the microscope, the basilar artery (BA) was dissected free from the brain stem, and was immediately immersed in cold (about 4^{o} C) Krebs solution (for composition, see below). The specimens were either used immediately or stored in a refrigerator at 4^{o} C for up to 24 h.

Recording of mechanical activity

Ring segments of the BA were mounted between metal prongs in organ chambers containing Krebs solution maintained at 37 ^{o} C (for methodological details: see Högestätt, Andersson & Edvinsson, 1983). The solution was continuously bubbled with 5 % CO_2 in O_2 resulting in a pH of approximately 7.4. When TRIS or HEPES-buffered solutions (for compositions, see below) were used the solutions were oxygenated with 100 % O_2. One metal prong was connected to a force-transducer for recording of isometric tension (Grass FT03C) and the other to a displacement device. The output was recorded on a polygraph (Grass 7C). The vessels were allowed to equilibrate for 60 - 90 min during which time they were subjected to a stepwise increase in passive tension, reaching a final level of about 5 mN.

Solutions

The following solutions were used:

1) "Normal Krebs" solution. This contained (in mM); NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11.0.

2) Depolarizing solutions containing 124 mM potassium (K^+). These were obtained by exchanging NaCl with equimolar amounts of KCl.

3) Calcium-free solutions. These were obtained by omitting $CaCl_2$ and adding 10^{-5} M EGTA (ehyleneglycol bis (β-aminoethylether)-N,N,N´,N´,-tetra-acetic acid).

4) TRIS or HEPES-buffered solutions. These were obtained by omitting $NaHCO_3$ and NaH_2PO_4, and adding either of 5 mM TRIS (tris(hydroxymethyl)-aminomethan) or 2.5 mM HEPES (N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid) and NaCl to maintain osmolality. The pH was adjusted by addition of either HCl or NaOH.

Drugs

$PGF_{2\alpha}$ was supplied as an aqueous solution (Amoglandin^R, Astra, Sweden) and was stored in the refrigerator until used. Manganese (Mn^{2+}) chloride (Sigma, USA) was dissolved in distilled water. Caffeine (Sigma, USA) was dissolved in hot distilled water at a concentration of 1 M. Diltiazem hydrochloride (Tanabe, Japan) was dissolved in distilled water and subsequent solutions were made up in 0.9 % NaCl. Nifedipine was supplied by Bayer AG (West Germany) dissolved in 15 % ethanol, 15 % polyethylene glycol and water (Adalat^R). The solutions were constantly protected from direct illumination in order to prevent degradation of nifedipine. Sialidase (neuraminidase, type X; Sigma, USA) was dissolved in distilled water at a concentration of 50 units/ml within 1 h before use. The solution was kept on ice until used. All concentrations given are the final concentrations of the active substances attained in the organ bath.

Experimental procedure

In all experiments two reproducible (accepted variation in maximum tension ±10 %) contractions were obtained with $PGF_{2\alpha}$ in normal Krebs solution. If not otherwise stated all subsequent contractile responses were expressed as % of the mean of these contractions. Relaxant responses were expressed as % reduction of the prevailing tension induced in the arteries by 2×10^{-5} M $PGF_{2\alpha}$ or 124 mM K^+.

When exposed to "calcium-free" medium, the arteries were incubated in nominally calcium-free Krebs solution containing 10^{-5} M EGTA. During the incubation-time the bath solution was exchanged at least once every five minutes and $PGF_{2\alpha}$ was subsequently added after twenty minutes in calcium-free solution. Contractions in calcium-free medium were elicited repeatedly without re-admission of calcium except in experiments with sialidase. In these

experiments the baths were rinsed twice after exposure to $PGF_{2\alpha}$ and the arteries subsequently exposed to sialidase (0.5 or 1 units/ml) for 30 min. After a brief (5 min) re-exposure to calcium (1.5 mM) the arteries were pretreated in calcium-free medium for 5 min after which time $PGF_{2\alpha}$ was added. At the end of each experiment, contractions were obtained with $PGF_{2\alpha}$ in calcium-containing solution.

Statistical analysis

Comparison between groups of data were performed by means of Student's two-tailed t-test. The 0.05 level of probability (p) was accepted as significant. Results are presented as mean ± standard error of the mean, n=number of experiments.

Results

Effect of calcium-free medium

After 20 min incubation in calcium-free medium, $PGF_{2\alpha}$ (2×10^{-5} M) induced a contraction, the maximum tension of which was 87 ± 4 % (n=42) of the contraction in calcium-containing Krebs solution. The response to $PGF_{2\alpha}$ was biphasic in all but one vessel segment; an initial, often partly non-sustained, contraction of low amplitude was followed by a second and sustained increase in tension (see e.g. Fig 2).

Effects of artificial buffers

With the intention to study the effects of calcium entry blockade with lanthanum (La^{3+}), experiments in TRIS and HEPES buffered solutions were attempted. However, in TRIS buffered solutions $PGF_{2\alpha}$ (2×10^{-5} M)-induced contractions were markedly attenuated as compared to contractions induced in Krebs solution (Table 1). K^+ (124 mM)-induced contractions did not significantly differ between bicarbonate-phosphate and TRIS buffered media; the contractions in Krebs solution amounted to 9.8 ± 0.9 mN (n=6) as compared to 11.4 ± 1.1 mN (n=6) in TRIS buffered solution. In HEPES-buffered solutions $PGF_{2\alpha}$-induced contractions did not significantly differ from contractions in Krebs solution (Table 1). However, $PGF_{2\alpha}$ failed to induce a biphasic response in eight out of nine preparations in calcium-free HEPES buffered solution; the contraction in calcium-free medium amounted to 15 ± 4 % of the control (n=9). Because of the divergent results with artificially buffered solutions and Krebs solution, no further attempts to utilize TRIS or HEPES buffered solutions were made. Manganese (Mn^{2+}) was used as an inorganic calcium entry blocker instead of La^{3+}.

Effects of calcium entry blockade with Mn^{2+} and diltiazem in calcium-free medium

Arteries contracted by 2×10^{-5} M $PGF_{2\alpha}$ in calcium-free solution (incubation for 20 min) relaxed in response to 1 mM Mn^{2+} by 75 ± 2 % (n=6). Nifedipine (3×10^{-6} M) induced a relaxation amounting to 87 ± 4 % (n=6). The maximum relaxation induced by Mn^{2+} was reached after 1 - 2 min but was consistently followed by a shortlasting

and slight increase in tension (Fig 1).

Pretreatment of the preparations with Mn^{2+} for 5 min abolished the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium. The first phase was slightly, but not significantly, increased (Fig 2, Table 2). Addition of nifedipine to arteries contracted by $PGF_{2\alpha}$ after pretreatment with both calcium-free solution and Mn^{2+}, had an inconsistent (four out of six experiments) relaxant effect of 7 ± 4 % (Fig 2).

Diltiazem-pretreatment (10^{-5} M for 15 min) eliminated the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium. The first phase was also significantly depressed (Table 2, Fig 2).

Effect of sialidase-pretreatment

After a control-contraction with $PGF_{2\alpha}$ (twenty minutes incubation in calcium-free medium) the preparations were exposed to 0.5 units/ml sialidase for 30 min. The vessels were thereafter exposed to 1.5 mM calcium for 5 min. After a subsequent five minutes incubation time in calcium-free solution, $PGF_{2\alpha}$ was added. The contractions thus obtained were compared with the responses to $PGF_{2\alpha}$ after 20 min incubation in calcium-free solution. The reason for the short (5 min) incubation after sialidase-pretreatment was to avoid emptying all cellular calcium-stores, assuming that the calcium-buffering capacity of the glycocalyx layer of the cell-membrane was eliminated (see Discussion).

The sialidase-pretreatment did not attenuate the first phase of the $PGF_{2\alpha}$-induced contraction but abolished the second in 3 out of 6 preparations. If the sialidase-concentration was increased to 1 unit/ml during the procedure described above, the second phase remained in one preparation only (Fig 3, Table 2). The ability of each segment pretreated with sialidase to respond to $PGF_{2\alpha}$ was ascertained by a contraction obtained in calcium-containing solution at the end of the experiment. These contractions were never attenuated as compared to the control contractions obtained with $PGF_{2\alpha}$ before calcium-exclusion and other manipulations.

Effects of caffeine

In calcium-containing Krebs solution, 20 mM caffeine elicited an

inconsistent (three out of five experiments) and shortlasting contraction, amounting to 14 ± 3 % of the $PGF_{2\alpha}$ (2×10^{-5} M) -induced contraction (n = 3), followed by a relaxation (Fig 4). After pretreatment in calcium-free medium for five minutes this contraction was further attenuated (Fig 4).

The $PGF_{2\alpha}$-induced contraction was almost abolished (n=5) during caffeine-exposure in calcium-free medium (Fig 4). After rinsing of the bath contents with calcium-free Krebs solution, $PGF_{2\alpha}$ induced a strong (64 ± 8 %, n=5) biphasic contraction (Fig 4). Pretreatment with 20 mM caffeine in calcium-free solution, followed by elimination of the drug, attenuated the first phase of the $PGF_{2\alpha}$ (2×10^{-5} M) -induced contraction. The second phase was not significantly affected (Fig 5, Table 2).

Caffeine (20 mM) completely relaxed arteries contracted by 2×10^{-5} M $PGF_{2\alpha}$ both in normal (104 ± 4%, n=4) and calcium-free (103 ± 1%, n=5) Krebs solution. Contractions induced by K^+ (124 mM) and a combination of K^+ and $PGF_{2\alpha}$ were also completely relaxed (103 ± 1%, n=5 and 98 ± 0,5%, n=4 respectively). Relaxation to below baseline-levels were consistently observed except in arteries contracted by K^+ and $PGF_{2\alpha}$ simultaneously.

Discussion

As shown previously (Uski & Andersson, 1984) $PGF_{2\alpha}$ induced a biphasic response in feline BA pretreated in calcium-free solution for 20 min. Similar responses were reported by $PGF_{2\alpha}$ in human pial arteries (Uski et.al., 1984) as well as by noradrenaline in the rat aorta (Heaslip & Rahwan, 1982). The biphasic contractile pattern could be reproduced several times in calcium-free medium without intervening re-exposure to calcium (Uski & Andersson, 1984; Uski et.al., 1984). Contractions induced by carbocyclic thromboxane A_2 in canine BA has also shown a similar independency of free extracellular calcium (Toda, 1982).

It has been shown that the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium can be abolished by nifedipine-pretreatment (Uski & Andersson,1984; Uski et.al., 1984). Since there are reports suggesting that nifedipine may have intracellular effects in addition to calcium entry blockade (Mikkelsen & Andersson, 1978; Church & Zsotér, 1980), the results with nifedipine-pretreatment alone do not allow any conclusions to be drawn concerning the possible location of the calcium-stores mediating the major second phase of the $PGF_{2\alpha}$-induced contraction. The second phase could, however, also be eliminated by increasing the EGTA-concentration in the extracellular medium to 10^{-4} M (Uski & Andersson, 1984; Uski et.al., 1984). In order to remove calcium bound to superficial intracellular stores, incubation in calcium-free solution containing 10^{-3} M EGTA is probably needed (Saida & vanBreemen, 1983). This, taken together with the proposal that nifedipine exerts its main action by blocking the "outer mouths" of the calcium channels (Glossmann et.al., 1982), made us to draw the conclusion that $PGF_{2\alpha}$ releases calcium bound to the exterior aspect of the cell membrane (Uski et.al., 1984).

The trivalent ion lanthanum (La^{3+}) is believed to act as a calcium antagonist by binding to negatively charged sites in the calcium channels (Weiss, 1974; Godfraind, 1981; Triggle & Swamy, 1983). In order to avoid precipitation of La^{3+}, experiments with artificially buffered solutions were attempted. However, in the present experiments both TRIS- and HEPES-buffered solutions had clear disadvantages, as has been described previously (Altura,

Altura, Carella & Turlapaty, 1980; Altura, Carella & Altura, 1980). It has actually been proposed that these buffers might have calcium-antagonistic properties (Altura, Altura, Carella & Turlapaty, 1980). In the present study TRIS attenuated $PGF_{2\alpha}$- but not K^+-induced contractions. This does not favour the view that TRIS acts as a calcium antagonist, since calcium entry blockers are believed to be more effective against depolarization-induced contractions than contractions induced by receptor-agonist interaction (Bolton, 1979; Godfraind & Miller, 1983) . Furthermore, HEPES did not depress $PGF_{2\alpha}$-induced contractions in calcium-containing solution, even if it clearly affected the response in calcium-free medium. Regardless of the mechanisms behind these effects of artificial buffers, they may gravely affect the results in studies on excitation-contraction (EC) coupling mechanisms.

Manganese (Mn^{2+}) also binds to negatively charged groups in the calcium channels and may, thus, be used as a calcium entry blocker (Steinsland, Furchgott & Kirpekar, 1973; Kostyuk, 1981; Nayler, 1983; Triggle & Swamy, 1983). However, it binds somewhat less tightly than La^{3+} (Kostyuk, 1981). Hence, the possibility that small amounts of Mn^{2+} might penetrate into the cell and replace Ca^{2+} in the EC-coupling process can not be entirely excluded. Nevertheless, pretreatment with Mn^{2+} in calcium-free solution eliminated the second phase of the $PGF_{2\alpha}$-induced contraction, as did the organic calcium entry blockers diltiazem, and, as shown previously, nifedipine (Uski & Andersson, 1984). Nifedipine did not consistently relax preparations contracted by $PGF_{2\alpha}$ after Mn^{2+}-pretreatment in calcium-free medium, a finding which does not support the view that nifedipine has more than, at most, marginal intracellular effects.

Pretreatment with Mn^{2+} did not attenuate the first phase of the $PGF_{2\alpha}$-induced contraction. However, depression of the first phase was seen after pretreatment with both diltiazem (this study) and nifedipine (Uski & Andersson, 1984). The finding that Mn^{2+} did not attenuate the first phase may indicate that Mn^{2+} penetrates into the cell and replaces calcium in the EC-coupling process, or that Mn^{2+} inhibits the calcium-extrusion from the cell.

The results with Mn^{2+} and diltiazem in the present study, as well as previous reports with nifedipine and high EGTA-concentrations, all suggest that a major part of the $PGF_{2\alpha}$-induced cerebrovascular contraction in calcium-free medium is mediated by the release of calcium bound to the outer face of the cell membrane. The view that contractions in cerebral arteries may depend on loosely bound "extracellular" calcium, has also been presented by others (McCalden & Bevan, 1981). Furthermore, results from ^{45}calcium flux determinations in the rat aorta suggest that contractions induced by the artificial prostanoid U44069 depend mainly on the release of calcium bound to the external surface of the plasmalemma (Loutzenhiser & vanBreemen, 1983). These possible binding sites may consist of membrane vesicles (Gabella, 1973; McGuffee & Bagby, 1976), binding sites in the lipid bilayer (Pang, 1980) or sialic acid in the glycocalyx layer (Langer, Frank & Philipson, 1982) of the cell membrane.

The second phase of the $PGF_{2\alpha}$-induced contraction was abolished in most preparations by pretreatment with sialidase. This enzyme has been shown to destroy sialic acid in the glycocalyx layer of the cell membrane, thereby reducing its calcium-binding capacity (Langer et.al., 1982; Rice & Webb, 1984). This would be expected to facilitate not only the removal of calcium bound to the membrane and stored in the membrane vesicles, but also intracellularly sequestered calcium. For this reason, the arteries were incubated in calcium-free medium for no more than 5 minutes after sialidase-pretreatment. The finding that the first phase was unaffected suggests that the intracellular calcium-stores were not exhausted.

In arteries briefly exposed to 20 mM caffeine the first phase of the $PGF_{2\alpha}$-induced contraction was markedly attenuated whereas the second was almost unaffected. There is evidence in favour of the view that caffeine at this high concentration releases most of the calcium bound to the sarcoplasmatic reticulum (Endo, 1977; Haeusler, Richards & Thorens, 1981; Saida, 1982; Saida & vanBreemen, 1983). Caffeine did not, however, completely abolish the first phase of the $PGF_{2\alpha}$-induced contraction. One explanation may be that caffeine actually does not release all intracellularly bound calcium. Due to its pronounced relaxant effects,

the failure of caffeine to induce more than a minimal contraction in calcium-free medium does not imply that it is incapable of exhausting intracellular calcium stores in the BA. In addition to releasing calcium from the sarcoplasmatic reticulum, caffeine has a variety of other effects e.g. phosphodiesterase-inhibition and effects on membrane polarity (Bolton, 1979). Such mechanisms may explain the present complex findings with caffeine.

In conclusion, the present study strongly supports the concept that a major part of the $PGF_{2\alpha}$-induced contraction in calcium-free medium is mediated via the release of calcium bound to the exterior aspect of the cell membrane.

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Table 1

Effects of artificial buffers on $PGF_{2\alpha}$- (2×10^{-5} M) induced contraction as compared to contractions in Krebs solution.

Buffer	Contraction in artificial buffer solution (mN)	Contraction in Krebs solution (mN)	n
TRIS	3.3 ± 1.1 [a]	6.7 ± 0.6	6
HEPES	11.2 ± 1.1	9.2 ± 1.0	5

a = $p < 0.05$

Table 2

Effects of pretreatment with various agents on the $PGF_{2\alpha}$-induced contraction in calcium-free medium.

Agent	Control Phase 1	Control phase 2	n	Pretreatment Phase 1	Pretreatment phase 2	n
Manganese (1 mM)	27 ± 4	77 ± 5	6	39 ± 3	2 ± 1 [b]	6
Diltiazem (10^{-5} M)	23 ± 4	70 ± 4	5	7 ± 1 [a]	≈0 [b]	5
Sialidase (0.5 units)	22 ± 4	71 ± 9	6	25 ± 1	29 ± 16	6
Sialidase (1.0 units)	20 ± 3	69 ± 11	5	24 ± 2	6 ± 11 [a]	5
Brief exposure to caffeine (20 mM)	25 ± 4	67 ± 8	6	11 ± 1 [a]	54 ± 12	6

Contractions are expressed as % of the contraction induced by $PGF_{2\alpha}$ in calcium-containing Krebs solution. Phase 2 was measured as the increase in tension from phase 1. Statistically significant results ($p < 0.01$ and 0.001) are indicated by [a] and [b], respectively

Fig 1 Relaxant effects of 1 mM manganese (Mn^{++}) and 3 x 10^{-6} M nifedipine (NIF) on $PGF_{2\alpha}$-contracted arteries in calcium-free medium.

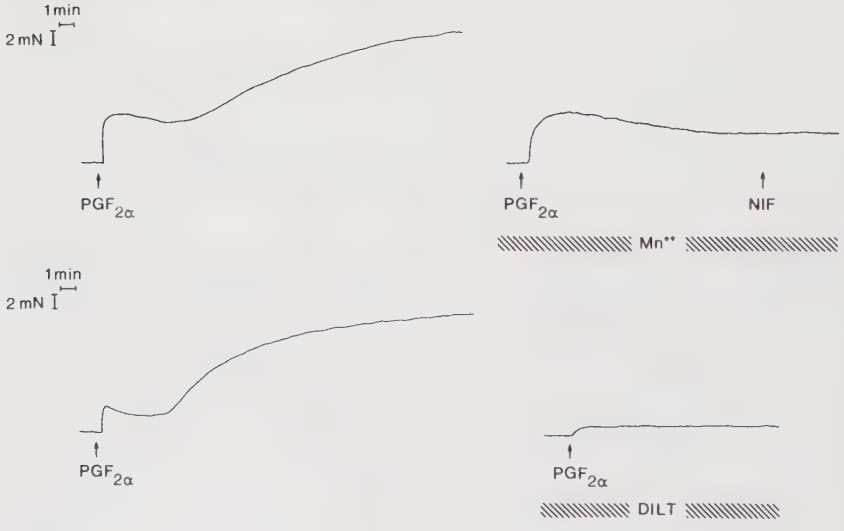

Fig 2 Tracings of contractions induced by 2 x 10^{-5} M $PGF_{2\alpha}$ in calcium-free medium demonstrating the effects of pretreatment with 1 mM manganese (Mn^{++}) or 10^{-5} M diltiazem (Dilt). NIF= addition of 3 x 10^{-6} M nifedipine.

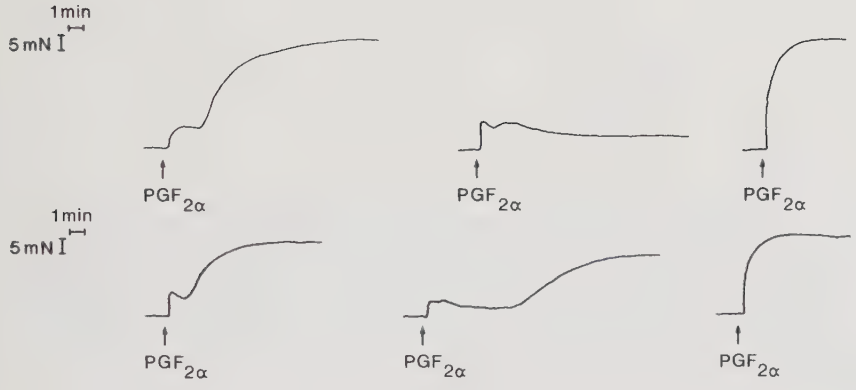

Fig 3 Effects of sialidase-pretreatment on contractions induced by 2×10^{-5} M $PGF_{2\alpha}$ in calcium-free medium. The tracings to the left were obtained after 20 min incubation in calcium-free medium, the middle after sialidase-pretreatment (0.5 units/ml for 30 min) and five minutes incubation in calcium-free medium. The tracings to the right are control contractions obtained in calcium-containing solution at the end of the experiments. The top tracings represent an experiment in which the second phase was eliminated by sialidase-pretreatment, whereas the other tracings illustrate an experiment in which the second phase was only slightly depressed and delayed.

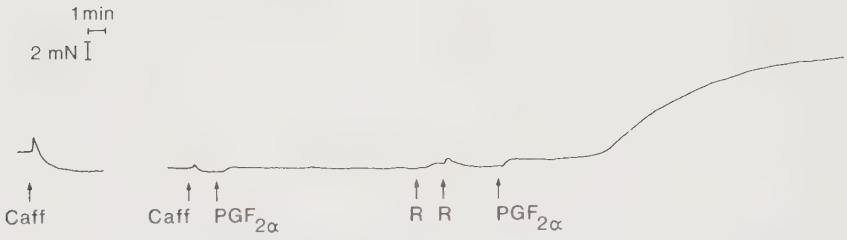

Fig 4 Tracings demonstrating the effects of 20 mM caffeine (Caff) and $PGF_{2\alpha}$ (2×10^{-5} M). The first tracing is obtained in calcium-containing solution and the succeeding ones during exclusion of free extracellular calcium. Note the reversible supression of the $PGF_{2\alpha}$-induced contraction in calcium-free medium.

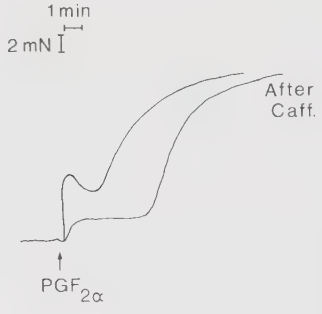

Fig 5 Contractions induced by $PGF_{2\alpha}$ (2×10^{-5} M) in calcium-free medium before and after a brief exposure to 20 mM caffeine (Caff).

VI

Some effects of the calcium promotor BAY K 8644 on feline cerebral arteries.

By

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Short title: Calcium entry and $PGF_{2\alpha}$ in brain arteries.

USKI, T.K. & ANDERSSON, K.-E. **Some effects of the calcium promotor BAY K 8644 on feline cerebral arteries.** Acta Physiol.Scand.

The proposed calcium promotor BAY K 8644 contracted feline basilar arteries partially depolarized by 10 mM potassium in a concentration-dependent manner (EC_{50}-value: (2.1±1.2) x 10^{-9} M). The concentration-response curve for prostaglandin (PG) $F_{2\alpha}$ was displaced to the left. $PGF_{2\alpha}$ induced a biphasic contraction in calcium-free medium. The second contraction phase was abolished by pretreatment with nifedipine or diltiazem. BAY K 8644 restored the second phase in arteries pretreated with nifedipine, but not in vessels pretreated with diltiazem. The findings suggest that BAY K 8644 acts as a promotor of calcium-influx, probably by interaction with the "dihydropyridine receptor" in the cell membrane, and also provide support for the view that $PGF_{2\alpha}$ releases membrane-bound calcium.

Key words: Calcium entry blockers, calcium promotor, calcium-free medium, $PGF_{2\alpha}$, feline basilar artery.

INTRODUCTION

Smooth muscle contraction is mediated by an increase of the free intracellular calcium-concentration, brought about by a release from cellular stores or by an influx through specific channels in the cell membrane (Bolton 1979). These calcium channels can be blocked by a variety of drugs, e.g. dihydropyridines such as nifedipine and nimodipine (Vanhoutte 1982, Triggle & Swamy 1983). Recently a dihydropyridine-derivative (BAY K 8644) with effects opposite those of e.g. nifedipine has been described (Schramm et al. 1983 a and b). It was proposed that this "calcium promotor" acts as a competitive antagonist to nifedipine (Schramm et al. 1983 a and b). To the best of our knowledge, there are no reports on the effects of this calcium promotor on cerebral arteries.

It has been demonstrated that prostaglandin (PG)-induced contractions in isolated canine, human and feline cerebral arteries are relatively independent of calcium in the extracellular medium (Toda 1982, Uski & Andersson 1984, Uski et al. 1984). $PGF_{2\alpha}$ induced a biphasic contraction of human and feline vessels pretreated in calcium-free medium: the major second phase was abolished by e.g. pretreatment with the calcium entry blockers nifedipine (Uski & Andersson 1984, Uski et al. 1984), diltiazem and manganese (Uski 1984), as well as by increasing the EGTA-concentration in the extracellular medium (Uski & Andersson 1984, Uski et al. 1984). All these findings support the concept that $PGF_{2\alpha}$ releases calcium bound to superficial parts of the cell membrane. This activator calcium must pass through the calcium channels in order to reach the contractile proteins. If this hypothesis is correct, it ought to be possible to modify the responses induced by $PGF_{2\alpha}$ in calcium-free medium with BAY K 8644.

The present study was performed in order to investigate the effects of BAY K 8644 on the feline basilar artery. Particular attention was given the the influence of this drug on the effects of $PGF_{2\alpha}$ in order to further elucidate the mechanisms by which this PG causes contraction in cerebral arteries.

MATERIAL AND METHODS

Adult cats were exsanguinated under sodium pentobarbital anaesthesia. The brain was removed and the basilar artery was dissected free from the brain stem. The vessels were immersed in cool Krebs solution (for composition: see below) and were either used immediately or stored at 4^{o} C for up to 24 h.

Ring segments of the arteries were suspended between metal prongs in 5 ml organ baths containing Krebs solution (for methodological details; see Högestätt et al. 1983), maintained at 37^{o} C. The solution was gassed with 95 % O_2 and 5 % CO_2 resulting in a pH of 7.4. One of the two prongs was connected to a force-transducer (Grass FT 03), and the output was recorded on a Grass polygraph. During an equilibration period of 60 - 90 min, the segments were subjected to a stepwise increase in tension, reaching a final stable value of about 5 mN.

The following solutions were used:

1. Normal Krebs solution which consisted of (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2 and glucose 11.0.

2. High potassium solutions with different concentrations of potassium (10 or 124 mM). These were prepared by an equimolar substitution of NaCl in the Krebs solutions for KCl.

3. Ca-free solution. This was identical with the normal Krebs solution except for replacing $CaCl_2$ with EGTA (10^{-5}M).

Nifedipine was supplied by Bayer AG (West Germany) dissolved in 15 % polyetyleneglycol, 15 % ethanol and water (Adalat^{R}). BAY K 8644 (Bayer AG, West Germany) was dissolved as nifedipine and was further diluted with 0.9 % NaCl. Both these drugs were protected against direct illumination. $PGF_{2\alpha}$, supplied as an aqueous solution (Amoglandin^{R}, Astra, Sweden), was diluted in phosphate buffer at neutral pH. Diltiazem hydrochloride (Tanabe, Japan) was dissolved in distilled water.

Concentration-response curves were obtained from experiments in which the drugs were added cumulatively to the organ baths. Preceding this, contractions were elicited with 124 mM K^{+} and all subsequent contractile responses were expressed as per cent of

the K^+-induced contraction. The drug concentrations eliciting half maximum response (EC_{50}) and the maximum responses (E_m) were determined.

In order to eliminate calcium in the extracellular medium, the arteries were incubated for 20 min in calcium-free solution containing 10^{-5} M EGTA, during which time the bath solution was exchanged every 5 min. Contractions were subsequently elicited repeatedly with $PGF_{2\alpha}$ without readmission of calcium, before and after pretreatment with nifedipine, diltiazem, and/or BAY K 8644. The responses in calcium-free medium were expressed as per cent of the mean of two reproducible (±10 %) contractions induced by $PGF_{2\alpha}$ in normal Krebs solution at the beginning of each experiment.

Student's two-tailed *t*-test was used for statistical comparison between groups of data. Whenever appropriate, results are given as mean ± standard error of the mean (SE), n=number of experiments.

RESULTS

In normal Krebs solution, BAY K 8644 (3×10^{-9} M) inconsistently contracted the preparations. In order to standardize the experimental conditions and to increase the sensitivity to the drug, the arteries were partially depolarized with 10 mM K^+. This induced a minimal (< 4 %) contraction in the vessels. In these partially depolarized vessels, BAY K 8644 in concentrations exceeding 3×10^{-10} M elicited concentration-dependent contractions (Fig 1). The concentration-response curve for BAY K 8644 was unaffected if $PGF_{2\alpha}$ (2×10^{-9} M) was also present (Fig 1). This $PGF_{2\alpha}$-concentration had per se almost no effect (see Fig 2). The EC_{50}-value for BAY K 8644 was $(2.1 \pm 1.2) \times 10^{-9}$ M without $PGF_{2\alpha}$ and $(2.6 \pm 1.2) \times 10^{-9}$ M with $PGF_{2\alpha}$ present. The E_m-values were 83 ± 2 % and 79 ± 2 %, respectively (n= 6 in both cases).

$PGF_{2\alpha}$ elicited concentration-dependent contractions in the feline BA (EC_{50}= $(3.6 \pm 1.2) \times 10^{-7}$ M: E_m= 100 ± 5 %, n= 5). If the arteries were pretreated with 3×10^{-10} M BAY K 8644 for 15 min, there was a left-ward shift of the concentration-response curve for $PGF_{2\alpha}$ (Fig 2), and the EC_{50}-value ($(7.4 \pm 1.2) \times 10^{-8}$ M, n= 5) was significantly ($p < 0.001$) lower than the control value.

$PGF_{2\alpha}$ (2×10^{-5} M) consistently induced a biphasic contraction (93 ± 3 % of the controls, n= 15) in arteries pretreated with calcium-free solution for 20 min (Fig 3). Incubation with BAY K 8644 (3×10^{-6} M for 10 min) in calcium-free medium transformed the biphasic contractile pattern into a contraction similar to that elicited by $PGF_{2\alpha}$ in calcium-containing solution in four consecutive experiments (Fig 3). Nifedipine-pretreatment (10^{-6} M for 15 min) in calcium-free solution abolished the second phase of the $PGF_{2\alpha}$-induced contraction. Subsequent addition of the calcium promotor BAY K 8644 restored this phase (Fig 4). Diltiazem (10^{-5} M) also abolished the second phase of the $PGF_{2\alpha}$-induced contraction. However, addition of BAY K 8644 (3×10^{-6} M) to these vessels did not restore the second phase. In nifedipine-pretreated arteries 88 ± 10 % (n=5) of the second phase was restored after BAY K 8644, as compared to 9 ± 7 % (n= 5) if diltiazem was used. This difference is statistically significant at the $p < 0.001$ level.

DISCUSSION

The proposed "calcium promotor" BAY K 8644 (Schramm et al. 1983 a and b) elicited contractions at very low concentrations in the feline BA partially depolarized by 10 mM potassium. This finding is in accordance with that described in other vascular preparations (Schramm et al. 1983 a and b), and supports the assumption that this drug promotes calcium-influx. It has been proposed that calcium channel blockers of the dihydropyridine-group, such as nifedipine, and the calcium promotor BAY K 8644 act on the same "dihydropyridine-receptor" in, or near, the calcium channels (Schramm et al. 1983 a and b). If this is true, cerebral arteries can be expected to be highly sensitive to BAY K 8644, since these vessels have been shown to be more susceptible than peripheral vessels to calcium channel blockade (Cauvin et al. 1983). This was also found: BAY K 8644 was a highly potent vasoconstrictor in the present cerebrovascular preparation.

The concentration-response curve for $PGF_{2\alpha}$ was shifted to the left after pretreatment with BAY K 8644 at a low concentration (3×10^{-10} M). This supports the view that BAY K 8644 may promote calcium influx in the feline BA, not only through potential sensitive channels (Bolton 1979), but also via channels opened by $PGF_{2\alpha}$. However, addition of 2×10^{-9} M $PGF_{2\alpha}$ to arteries partially depolarized by 10 mM potassium did not displace the concentration-response curve for BAY K 8644 to the left. This may be due to the concentration of $PGF_{2\alpha}$ being too low. On the other hand, at higher concentrations $PGF_{2\alpha}$ had contractile effects per se, which would have complicated the interpretation of the results obtained with such concentrations.

After pretreatment of the arteries in calcium-free medium, $PGF_{2\alpha}$ induced a biphasic response as described previously (Uski & Andersson 1984, Uski et al. 1984, Uski 1984). After pretreatment with BAY K 8644, this contractile pattern was transformed into a response similar to the contraction induced by $PGF_{2\alpha}$ in the presence of calcium in the extracellular medium. Furthermore, the second phase, which was abolished by nifedipine-pretreatment, was restored after addition of BAY K 8644. In contrast, the second phase was not restored by this drug, if it had been abolished by pretreatment with diltiazem. This supports the view proposed by

Schramm et al. (1983), that BAY K 8644 and nifedipine, but not diltiazem, may act on a common "dihydropyridine receptor" regulating the influx of calcium through the membrane channels. The differences between nifedipine and diltiazem are also consistent with the proposal by Glossmann et al. (1982) that different calcium entry blockers have different sites of action. If BAY K 8644 indeed acts at the membrane level by stimulating calcium-influx, the present findings further support the concept that $PGF_{2\alpha}$ releases calcium from stores located on the exterior expect of the cell membrane (Uski & Andersson 1984, Uski et al. 1984, Uski 1984).

In conclusion, BAY K 8644 seems to be a potent "calcium promotor" in feline BA and, thus, a useful tool in research on the excitation-contraction coupling in the cerebrovascular bed. It may also promote PG-induced calcium influx. The present findings provide further support for the view that $PGF_{2\alpha}$ is able to release calcium bound to superficially located membrane stores.

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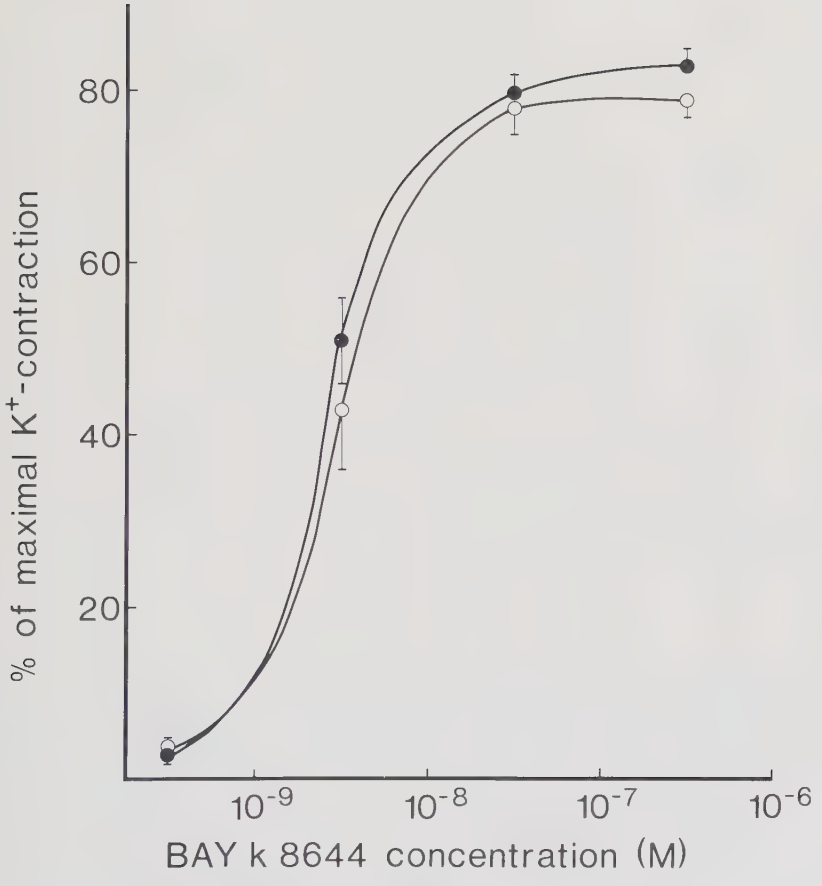

Fig 1 Effects of BAY K 8644 on arteries exposed to 10 mM K^+ (●), or 10 mM K^+ and 2 x 10^{-9} M $PGF_{2\alpha}$ (o). The responses are expressed as % of the contraction induced by 124 mM K^+.

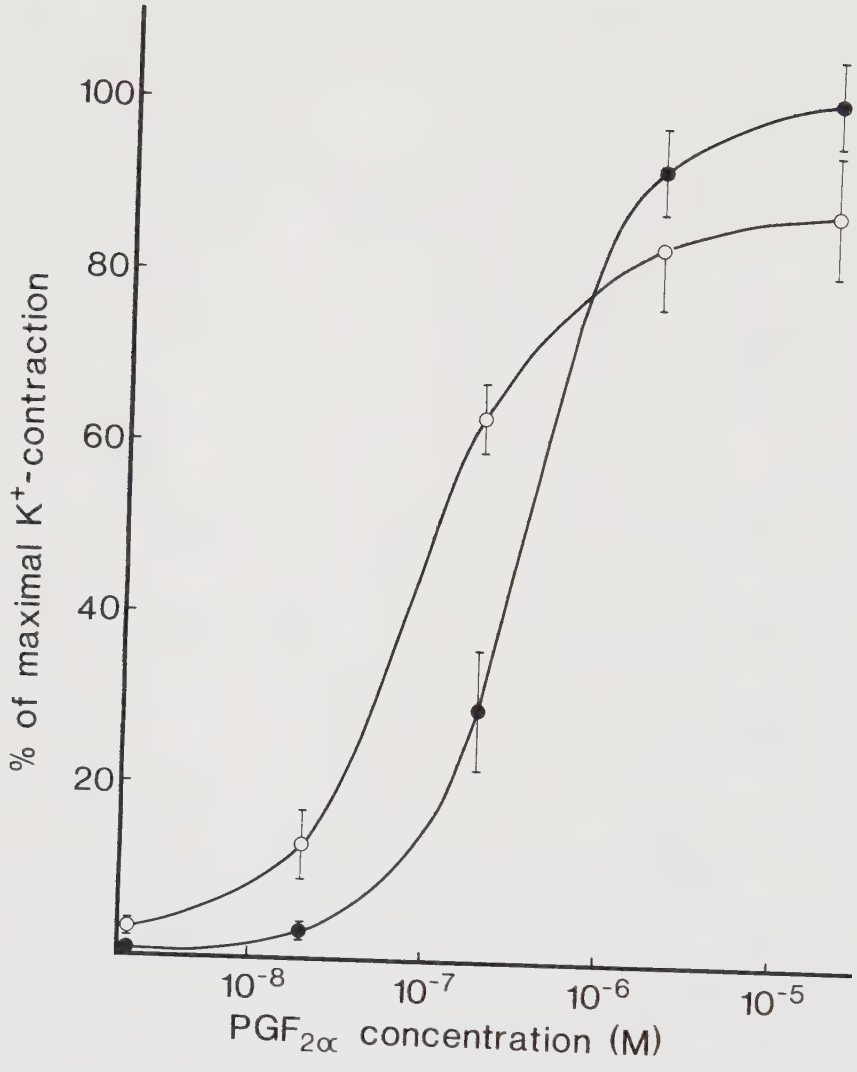

Fig 2 Concentration-response curves to $PGF_{2\alpha}$ in the absence (●) or presence (o) of BAY K 8644 (3×10^{-10}M). The responses are expressed as % of the contraction induced by 124 mM K^+.

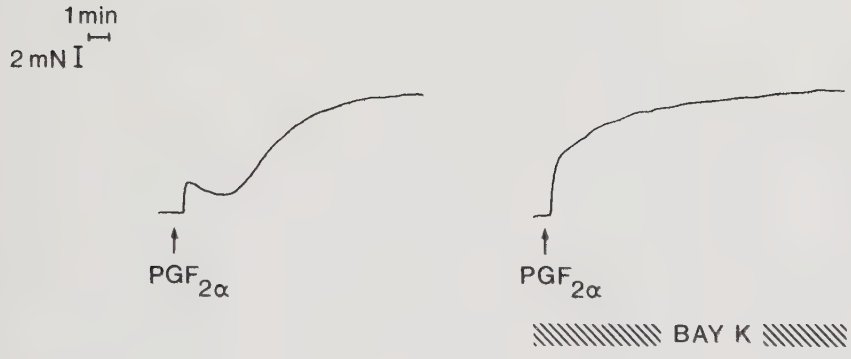

Fig 3 Tracings illustrating the effect of BAY K 8644 on the contraction obtained with $PGF_{2\alpha}$ in calcium-free medium. The two tracings were obtained in the same preparation without intervening calcium-exposure.

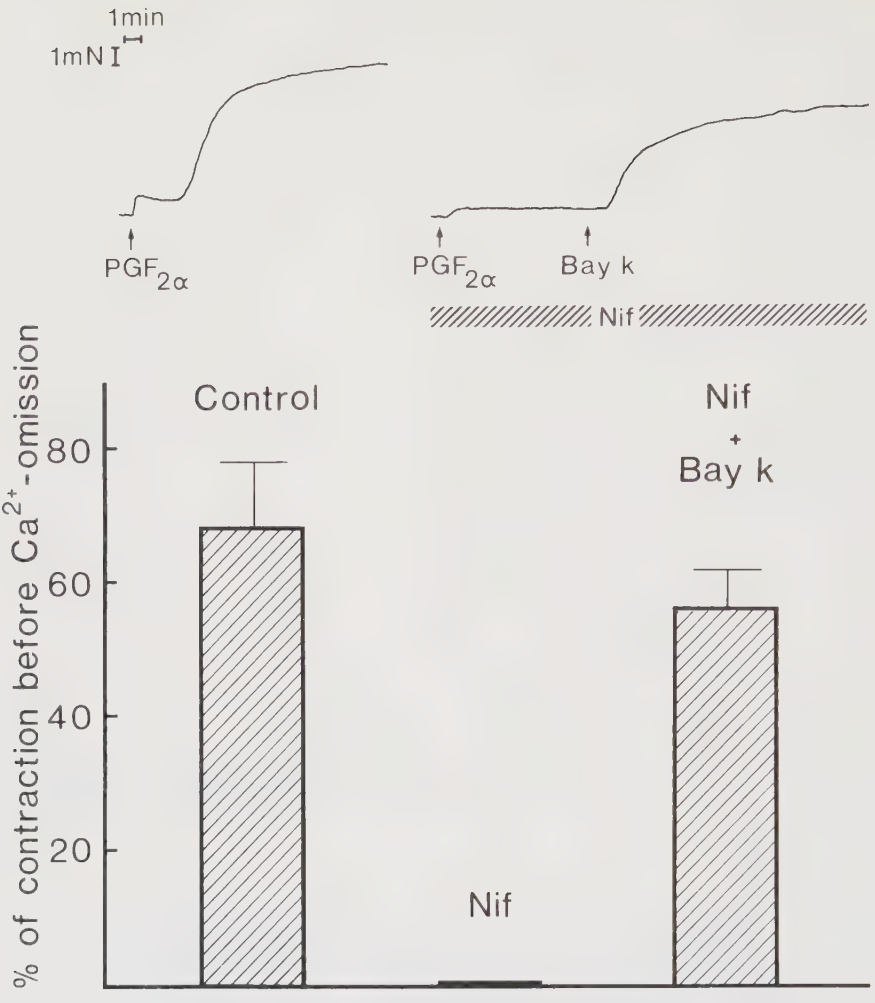

Fig 4 Effects of nifedipine (10^{-6} M) and a combination of nifedipine (Nif) and the "calcium-promotor" BAY K 8644 (3 x 10^{-6} M) on the amplitude of the second phase of the $PGF_{2\alpha}$-induced contraction in calcium-free medium (n=5). The contractions are expressed as % of the contraction obtained with $PGF_{2\alpha}$ in calcium containing solution. The inset shows representative tracings demonstrating the effects of nifedipine and BAY K 8644 on the contractile response obtained with $PGF_{2\alpha}$ in calcium-free solution.

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